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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held at University College, Gower Street, London, W.C.* 1, *on* 22 *March* 1956, *at* 10.45 *a.m., when the following papers were read:*

COMMUNICATIONS

The Composition of Some Protein Fractions Extracted from Calf Skin. By J. H. Bowes, R. G. Elliott and J. A. Moss. (*British Leather Manufacturers' Research Association, Egham, Surrey*)

During investigations on the extraction of acid-soluble collagen from skin, indications of the presence of a non-collagenous protein constituent closely associated with the collagen were obtained (Harkness, Marko, Muir & Neuberger, 1954; Bowes, Elliott & Moss, 1955). Further work on the exhaustive extraction of calf skin with dilute acid and alkaline solutions has now furnished evidence for the presence of two non-collagenous protein fractions in this tissue.

The middle layer of a calf skin was disintegrated and extracted successively with six changes of each of the following solutions: phosphate buffer, pH 9·1; citrate buffer, pH 3·7; dilute acetic acid, pH 2·8; and sodium hydroxide, pH 12·2. The bulk of the protein extracted by the dilute acid solutions was precipitated by sodium chloride, and hydroxyproline and tyrosine determinations indicated that it was mainly collagen. The filtrates were dialysed and concentrated. The composition of the two protein fractions so obtained differed considerably from that typical of collagen; in particular, the hydroxyproline content was negligible, the glycine, proline and arginine contents were lower, and the tyrosine, histidine, leucine, isoleucine, valine, dicarboxylic acids and amide contents were higher than in collagen. The composition of the two fractions differed slightly, but there was insufficient evidence to decide whether they were two different proteins, or different degradation products of the same protein.

The fraction isolated from the alkaline extracts by precipitation with ethanol was also found to consist mainly of non-collagenous-type protein. Its composition was similar to those of the acid-soluble non-collagenous fractions, but there were certain differences. Both the acetic acid-soluble and the alkali-soluble fractions were associated with relatively large amounts of hexosamine.

Thus, two relatively soluble non-collagenous proteins appear to be closely associated with the collagen in skin. The alkali-soluble fraction may represent the protein moiety of the mucopolysaccharide of the ground substance. The hydroxyproline content of the extracted calf skin indicated that it still contained about 7 % of a non-collagenous protein. This protein fraction must be closely associated with the collagen and may even form an integral part of the collagen fibres. It is possible that the acid-soluble non-collagenous fractions represent soluble forms of this component.

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The Biosynthesis of Ergothioneine by *Claviceps purpurea*. I. The Incorporation of [2-^{14}C]-Acetate. By H. Heath and Jennifer Wildy. (*University College Hospital Medical School, London, W.C.* 1)

Claviceps purpurea, growing parasitically on rye, forms sclerotia which contain alkaloids and ergothioneine. This fungus has been grown on numerous solid and liquid media; in no case have sclerotia been formed and neither alkaloids nor ergothioneine could be detected. After repeated subculturing, ergothioneine was formed in cultures grown on a mannitol, ammonium succinate, sulphate, phosphate and trace metal medium and was labelled by the addition of 1·0 mc [2-^{14}C]acetate. Ergothioneine was isolated by ion exchange and alumina (Melville & Horner, 1953) chromatography and crystallized to constant activity. It was degraded as follows: ergothioneine, trimethylamine and thiolurocanic acid; thiolimidazole propionic acid; imidazole propionic acid; ethyl imidazole propionate; ethyl 4:5-dibenzamidopentenoate and sodium formate; carbon dioxide and

3,4-dibenzamidobutene. All compounds except the last, which was not isolated, were converted to $BaCO_3$ prior to counting.

The highest activity (2411 c.p.m./mg. C) was present in C-2 of the ring, followed by the methyl carbons (500 c.p.m./mg. C) of the betaine; the five-carbon residue had only 113 c.p.m./mg. C, the carboxyl carbon accounting for at least 60% of this activity. The low activity of the five-carbon chain of ergothioneine shows that glutamate, derived from acetate via the citric acid cycle, is not the precursor of this chain. Thus there are similarities between the pathways of histidine and ergothioneine biosynthesis.

At present, the precursors of the five-carbon chain in histidine are not definitely known. Glutamate is not the precursor as [2-^{14}C]acetate is not incorporated into this chain by yeast (Levy & Coon, 1954). Ehrensvard, Reio, Saluste, & Stjernholm (1951) showed, however, that with yeast grown on acetate as the sole carbon source the carboxyl carbon of histidine is derived from the methyl carbon of acetate.

Support for a pentose pathway is given by Ames & Mitchell (1955), who showed that histidine mutants of *Neurospora* accumulated imidazole-glycerol, imidazole-acetol and histidinol.

Work is in progress to see whether the formation of the thiolimidazole moiety occurs by addition of sulphur to the preformed imidazole ring system or whether the sulphur is attached to the ultimate C-2 of the ring before ring closure occurs.

The authors are very grateful to Messrs Burroughs Wellcome Ltd for a gift of ergothioneine.

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Detection of Tissue Clearing Factor by its Effect on Lipoprotein Electrophoresis. By A. Comfort. (*Department of Zoology, University College, London, W.C.* 1)

Korn (1955) has found that normal tissues, and acetone powders prepared from them, contain a substance which appears identical with the clearing factor released into the plasma by injected heparin. We have used the selective increase in the electrophoretic migration of serum lipoproteins to detect response to heparin in lipaemic rabbit serum, which does not undergo clearing (Comfort, 1955). This is a sensitive test either for clearing factor or for something closely associated with it.

Fresh brei and acetone powders from several tissues increase the migration of added plasma lipoprotein. Tissues were minced with scissors; powders were prepared by homogenizing in acetone at $-20°$ and drying at room temperature. Human plasma was used as substrate. Each batch was divided into many 5 ml. portions which were frozen at $-20°$ and thawed once only as required. Fresh brei or acetone powder was ground with 2 ml. test plasma, centrifuged after 2 hr. at room temperature, and examined electrophoretically, after dilution with more test plasma if necessary. A rough quantitative estimate of activity was given by comparison with standards.

Lipoprotein migration was selectively increased by fresh and acetone-dried mouse, rabbit and duck heart and lung, fresh rabbit kidney and lymph gland, but not by skeletal muscle or frog lung, liver, heart, testis, caecum or muscle.

Korn (1955) extracted clearing factor from acetone powders with serum or 0·025N-NH_4OH. Although activity detectable both by optical clearing and by lipoprotein acceleration is extracted by alkali, the bulk of the activity, judged by the second test, remains attached to the solid residue. This does not indicate that tissue factor is particulate, since it is equally hard to elute from alcohol precipitates of serum proteins. The identity of clearing and accelerating substances cannot be assumed, but they show no tendency to separate.

We found no evidence of interference by other tissue constituents. Comparison of parallel runs with successive dilutions of the factor indicates that it produces a simple increase in the anodic migration of one or both lipoprotein bands, not a transfer of lipoid from one serum protein to another (Brown, Boyle & Anfinsen, 1953). Action on the α-lipoprotein is inconstant—albumin mobility is sometimes increased, probably as a result of the binding of fatty acids released by the factor or an accompanying lipase (Ballou, Boyer & Luck, 1945).

This work was done under a Fellowship awarded by the Nuffield Foundation.

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The Chemical Nature of Tetrahydroxynorsterocholanic Acid. By G. A. D. Haslewood (*Guy's Hospital Medical School, London, S.E.* 1) and I. D. P. Wootton (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The above acid was isolated by Ohta (1939) from the bile of the Japanese 'Gigi' fish, and since it has been found in the bile of other teleostean fish and also in that of chickens (Yamasaki, 1951), it is probably a 'primitive' bile acid of widespread occurrence.

Ohta (1939) and Isaka (1940) after a study of its chemistry concluded that tetrahydroxynorsterocholanic acid, $C_{27}H_{46}O_6$, was a 3:6:12:24-tetrahydroxy 'coprostanic acid', and could be degraded to 3:6:12-trihydroxycholanic acid. Attempts at partial synthesis of 3α:6α:12α-trihydroxycholanic acid have given material not having the properties mentioned by Isaka (1940) and we decided to test by direct examination the formula of Ohta and Isaka. A sample of tetrahydroxynorsterocholanic acid, m.p. 215–217°, kindly given by Professor T. Kazuno of Okayama Medical School, was methylated and examined by infrared spectroscopy. In the region 900–1300 cm.$^{-1}$, the spectrum closely resembled that of methyl cholate (3α:7α:12α-trihydroxycholanate). According to observations previously made (Wootton, 1953), we conclude that tetrahydroxynorsterocholanic acid almost certainly has the cholic acid nucleus, i.e. it resembles all other 'primitive' bile acids or alcohols so far examined in being a 3α:7α:12α-trihydroxycholane derivative.

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The Speed of Oxidation of Reduced Nicotinamide Nucleotides by Cerebral Preparations. By H. McIlwain and M. A. Tresize. (*Department of Biochemistry, Institute of Psychiatry, British Postgraduate Medical Federation, University of London, Maudsley Hospital, London, S.E.* 5)

A recent survey of respiration in cerebral systems (McIlwain, 1955) showed known rates of oxidation of reduced nicotinamide nucleotides to be collectively slower than the maximal rate of respiration of the brain or of separated cerebral tissues. The rates have accordingly been re-examined.

Optimal conditions for measuring cytochrome *c* reductases, or the oxidation of reduced cozymase, have been found to include histidine or a surrogate. Rates now observed reach 1000 μmoles nucleotide oxidized/g. tissue/hr. They are sensitive to the concentration of reduced cozymase at levels up to 150 μM. As the reduced cozymase levels in rat brain (Glock & McLean, 1955) correspond to 130 ± 55 μmoles/g., control of respiration in part through the level of reduced coenzymes is feasible.

Reduced 2′-phosphocozymase was relatively slowly oxidized in the presence of cerebral tissues and cytochrome *c* or oxygen, but much more rapidly with glutathione (Rall & Lehninger, 1952). Rates up to 200 μmoles nucleotide/g. tissue/hr. have now been observed, again considerably above previous observations. Reduced cozymase was oxidized only slowly in this system. Other indications of the distinctness of systems metabolizing the two coenzymes have been obtained.

The rates now observed are collectively much greater than the tissues' maximal rate of respiration, which is about 300 μatom O or μmoles reduced nucleotide/g. tissue/hr. The rates of oxidation of reduced cozymase are not uniform in different parts of the adult brain; they are not highest in the parts of greatest respiratory rate in the adult animal but, curiously, in those of greatest rate at an earlier stage of development.

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A Benzoic Acid Adsorption Method for Extracting Parathyroid Hormone from Human Urine. By Beryl M. A. Davies and Russell Fraser. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Evidence that parathyroid hormone (P.T.H.) can be excreted in mammalian urine was obtained from experiments with mice injected with 'Parathormone' (Eli Lilly Co.) labelled with ^{131}I by a modification of Talmage, Dixon, Bukantz & Dammin's (1951) method.

Attempts were therefore made to extract the hormone quantitatively from human urine. 'Parathormone', or ^{131}I-labelled 'Parathormone', was added to urine, after the destruction of endogenous hormone by HCl. After extraction, hormone recovery was tested either by a mouse urine

phosphate assay (Davies, Gordon & Mussett, 1955) or by radioactivity measurements, assuming that high ^{131}I recovery indicated high hormonal recovery.

Three extraction procedures, ultrafiltration and adsorption on aluminium hydroxide or benzoic acid, were investigated and only the latter led to a satisfactory method. In this, 48 hr. urine, refrigerated as passed, was adjusted to pH 3·5. At 0°, 10 ml. of saturated benzoic acid in absolute ethanol were added per 100 ml. urine (except in diuresis, when proportionately less benzoic acid was used). The precipitate formed (precipitate A) was dried. Originally, the benzoic acid was dissolved by three washings with absolute ethanol (2·7 l.) at 0°. The residue (precipitate B), containing hormone, was dried. Bioassay and radioactivity measurements showed that about 80 % of the added hormone was recovered. P.T.H. activity is not lost by boiling at an acid pH: neither was that of precipitate B.

Assuming 'Parathormone' to contain 100 phosphate units/ml., human urinary excretions of P.T.H. (units/24 hr.) were:

Normal subjects (4): average 67, range 61–71.

Confirmed hyperparathyroid patient (uraemic): 139.

Re-extraction of above hyperparathyroid urine: *ca.* 16.

Hypoparathyroid patient: <30.

For six normal subjects, the average weight of precipitate B from 48 hr. urines was 1·0 g. Biuret estimations showed the average protein content to be 0·59 g.; part of the residue was benzoic acid. Attrition of precipitate A and five ethanol washings reduced the weight of precipitate B in two experiments to 0·3 g. On bioassay, parathyroid activity was similar to that obtained in the original procedure. Further purification of precipitate B is being attempted in order to increase the solubility of this product and so facilitate animal testing.

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The Fluorimetric Estimation of Adrenaline, Noradrenaline and Hydroxytyramine in Urine.

By H. Weil-Malherbe. (*Runwell Hospital, Wickford, Essex*)

If the methods of Weil-Malherbe & Bone (1952, 1953) and Lund (1949, 1950) for the fluorimetric estimation of adrenaline and noradrenaline are compared, it is found that both give approximately the same result when applied to plasma (Weil-Malherbe, 1955), but that the former gives much higher results than the latter with acid-hydrolysed urine. This is due to the presence of hydroxytyramine (von Euler, Hamberg & Hellner, 1951), 3:4-dihydroxyphenylacetic acid (von Euler, von Euler & Floding, 1955) and possibly other acidic catechols in hydrolysed urine. These substances form fluorescent derivatives with ethylenediamine, but do not interfere with the methods based on the formation of 3:5:6-trihydroxyindole (Lund, 1949).

Acidic and basic catechol compounds could be separated by either anion or cation exchange. The basic fraction is obtained in a high degree of purity by adsorption on a cationic resin in the H^+-form (Zeokarb 225, water regain 1·8, 40–70 mesh) and elution with 0·2 M-$BaCl_2$. This resin treatment is preceded by passage of the urine through a column of alumina at pH 8·5 and elution of the catechol fraction with acid.

The purified fraction of catecholamines has been investigated by paper chromatography and bioassay. The three catecholamines were found to be the only constituents giving rise to yellow-green fluorescence with ethylenediamine. It is therefore possible to estimate the sum of the three catecholamines by the ethylenediamine method, using a yellow filter. Adrenaline and noradrenaline are estimated separately by the method of Lund (1949, 1950) or of von Euler & Floding (1955). Hydroxytyramine is obtained by difference.

Recoveries of added catecholamines varied from 60–110 %, mean 87 %. The concentrations found in 30 samples of acid-hydrolysed urine were (means ± S.E.): adrenaline $11·2 \pm 0·89$, noradrenaline $24·8 \pm 1·68$, hydroxytyramine $82·3 \pm 13·3$ μg./l.

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Some Aspects of the Reactions of D-Glucose with Glutamic Acid, Valine and Lysine. By S. LEWIN and C. FOX. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The reactions of glucose with valine, lysine and glutamic acid have been noted (Agren, 1940; Kubota, 1941), but their mechanism has apparently not been investigated. The 'equilibrium' constant of the valine–glucose interaction at 30° was given as 5·5 (Katchalsky & Sharon, 1953) on the assumption that the reaction involves one molecule of glucose and one molecule of the valine anion. Preliminary investigation of the glutamic acid–glucose reaction (S. Lewin & D. A. T. Southgate, unpublished observations) showed, however, that the monoglucose mechanism cannot explain the data obtained.

We have examined these reactions systematically, aerobically and anaerobically, using varying amino acid concentrations (0·1 to 0·0143M), initial pH values (7–9), glucose concentrations (up to 1·2M) and temperatures (20–50°), using techniques and apparatus (Lewin, 1953, 1955*a*) similar to those reported in the study of histidine–glucose interaction (Lewin, 1955*b*, 1956).

The phenomenon of consecutive steady pH states originally observed in the histidine–glucose reaction was encountered in all the three amino acid–glucose interactions. The results obtained in the first steady pH state require the recognition of the coexistence of both monoglucose and diglucose reversible interactions with one molecule of the amino acid anion involved. The overall three reactions, in the first steady pH state, were found to be exothermic. These results are parallel to those obtained in the alanine–glucose and glycine–glucose interactions (Lewin & Kosinski, 1956).

The respective aerobic pH depression values were practically the same as those obtained anaerobically at low temperature and low glucose concentrations; but with rise in temperature, glucose concentration and steady pH state number, the aerobic ΔpH values recorded were noticeably higher than the anaerobic values.

Decreasing amino acid concentrations in the glutamic acid–glucose and lysine–glucose interactions, other things being equal, resulted in some increase in the pH depression values. But this phenomenon was noted in the valine–glucose interaction only at 50°.

The addition of urea (up to 2·94M) NaCl and Na_2SO_4 (up to 0·368M) did not affect the pH depression values.

Browning of the solutions was noted in all the cases examined, and the colour intensities developed were parallel to those observed in the histidine–glucose interaction. But the aerobic inhibition of the colour development was far more pronounced, resulting in some cases in total suppression.

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Some Aspects of the Reaction of L-Histidine with D-Ribose, D-Xylose, Fructose, Glucose and Lactose. By S. LEWIN. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The phenomenon of consecutive steady pH states observed originally in histidine–glucose solutions (Lewin, 1955, 1956) and later in lysine–glucose, valine–glucose, glutamic acid–glucose (Lewin & Fox, 1956), glycine–glucose and alanine–glucose solutions (Lewin & Kosinski, 1956) has now been found with other systems such as histidine–ribose, histidine–xylose, histidine–fructose and histidine–lactose.

The times required for the attainment of, and the lifetimes of, the steady pH states were much shorter in the pentose–histidine than in the hexose–histidine interactions. Thus at 20° glucose–histidine solutions required about a week for the attainment of the first steady pH state—which lasted for about 4 weeks—while the corresponding pH state in ribose–histidine solutions was attained in about an hour and lasted only about 3 days; the respective 'equilibrium' constants were, however, not greatly different, e.g. 5·0 for the ribose–histidine and 4·3 for the xylose–histidine compared with 3·9 for the glucose–histidine reaction. The first two values have been obtained over the concentration range up to 0·6M pentose where the results are in agreement with the reversible reaction of one pentose molecule with one molecule of the histidine anion.

The increase in the pH depression values with decrease in amino acid concentration, noted in the last three references, has been investigated further in the histidine–glucose and histidine–fructose reactions, and has been found to be more pronounced, particularly in histidine–fructose solutions, with histidine concentrations below 0·0143M. In some cases the increased effect was so great that no steady pH states could be noted, a continuous increase in the pH depression values being obtained instead.

The development of brown colour, as associated with the 'browning reaction', was observed in all the histidine–monosaccharide solutions investigated. But, under similar conditions, fructose, ribose and xylose developed greater colour intensities than the corresponding glucose solutions. However, in the case of the xylose–histidine interaction a variation was noted in that the initial colour developed was distinctly green at first; gradually, however, the solution browned to such an extent that no green tint was visible.

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The Excretion of Bilirubin as an Ester Glucuronide, Giving the Direct van den Bergh Reaction.

By BARBARA H. BILLING and G. H. LATHE. (*Research Laboratories, Queen Charlotte's Maternity Hospital, London, W. 6, and the Institute of Obstetrics and Gynaecology, University of London*)

The excretion of bilirubin involves its conversion to an unstable substance (pigment II of Cole, Lathe & Billing, 1954). Unlike bilirubin, it is water soluble, and gives a direct van den Bergh reaction. This transformation has been investigated by comparing the two dipyrryl azopigments (A and B) which are formed on coupling bilirubin and pigment II with diazotized aniline (Billing, 1954). One molecule of bilirubin yields 2 molecules of pigment A (Overbeek, Vink & Deenstra, 1955), while pigment II produces the more polar pigment B.

Fresh human hepatic bile was collected by fistula, and contained mainly pigment II. Diazotized aniline was added. Pigment B was salted out with ammonium sulphate, extracted with methanol, and freed of lipid, pigment A, and conjugated bile acids, by a series of counter-current distributions. It was purified by precipitation from methanol–ethanol (1:5) at $-12°$. Pigments A and B had similar absorption spectra, but on a weight basis B had a smaller extinction. Mild alkaline hydrolysis of pigment B yielded pigment A and a reducing substance, which moved and stained like glucuronic acid when chromatographed on paper. Quantitative analysis (Fishman & Green, 1955) showed that in pigment B the molar proportion of glucuronic acid to pigment A was 1, and together they accounted for 87% of the dry weight of the purest preparation of pigment B.

β-Glucuronidase (mouse liver, locust crop juice) converted pigment B to A. Because of its alkaline lability it seems probable that pigment B is an ester glucuronide of pigment A. Accordingly the parent substance, pigment II, might be either the mono- or di-glucuronide of bilirubin. The glucuronic acid content of bile is usually insufficient to account for the conjugation of both carboxyl groups of all the diazotizable pigments. However, the yield of pigment B from impure pigment II suggests a di-glucuronide structure.

Alkali treatment of hepatic bile changed the usual direct van den Bergh reaction to an indirect one, characteristic of bilirubin. Sera from patients with obstructive jaundice and hepatitis, containing direct reacting pigments, had increased amounts of alkali labile glucuronides.

It seems probable that the main pathway of bilirubin excretion in the human is through conjugation with glucuronic acid.

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Excretion of Cystathionine in Pyridoxine Deficiency. By H. Blaschko and D. B. Hope. (*Department of Pharmacology, University of Oxford*)

In chromatograms of urine from rats reared on a diet deficient in pyridoxine, a ninhydrin-positive spot appears which is not present in the urine of litter-mates receiving adequate vitamin B_6 (Blaschko, Datta & Harris, 1953). This spot was provisionally considered to be phosphorylethanolamine.

The unknown spot has now been identified as L-cystathionine. When phosphorylethanolamine was added to urine, two closely adjoining but distinct spots were seen in the chromatograms, but upon addition of L-cystathionine there was only one spot. The unknown spot gave the test with platinic iodide characteristic of S-compounds (Winegard, Toennies & Block, 1948). In the solvent system described by Hoare & Work (1955) the L and D isomers of cystathionine could be separated; the unknown substance appeared in the position of the L isomer. The unknown compound behaved like cystathionine on treatment with Raney nickel (Berridge, Newton & Abraham, 1952). Professor D. D. Woods kindly investigated the activity of the compound with mutants of *Esch. coli* of the methionine series and found its properties consistent with those of cystathionine.

The mammalian enzyme that splits cystathionine is known to be a pyridoxal protein. Our experiments show that B_6 deficiency causes a metabolic block; cystathionine, normally an intermediary metabolite, appears as an end-product of metabolism.

We are grateful to Dr Datta for the phosphorylethanolamine and to Professor Du Vigneaud for the isomers of cystathionine.

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A Comparison of Manometric Methods of Estimating Hydrogenase with the Deuterium Exchange Method. By T. Kameyama, Y. Kondo and N. Tamiya. (*Chemistry Department, University of Tokyo, and Biochemistry Department, University of Oxford*)

As shown previously (Tamiya, Kameyama, Kondo & Akabori, 1955), hydrogenase activity in bacteria can be estimated by following manometrically the evolution of hydrogen in the presence of reduced methylviologen. This method differs from the usual method for estimating this enzyme by hydrogen uptake in the presence of a hydrogen acceptor. It appeared worthwhile to compare these different manometric methods with the deuterium exchange method (Hobermann & Rittenberg, 1943), which is believed to measure hydrogenase activity directly. The final method for using methylviologen was as follows: the enzyme preparation, 0·25 M phosphate buffer at pH 7·0, and 0·0033 M methylviologen were placed in the main compartment and 6 mg. sodium dithionite was placed in the side arm of the Warburg vessel. Alkaline pyrogallol and paper were placed in the centre well; the gas phase was nitrogen. After 10 min. equilibration at 30°, the dithionite was mixed with the enzyme and the hydrogen production followed manometrically. The rate was linear until all the dithionite was oxidized. When the rate of hydrogen production was less than 2000 μl./hr. the rate was proportional to the enzyme concentration. This is in contrast to the methylene blue method where the rate of hydrogen uptake is generally not proportional to the enzyme concentration. The methylviologen method thus shows no dilution effect. The deuterium exchange experiment was carried out in a specially designed flask and HD in the gas phase was estimated in a mass spectrometer. Na dithionite was also added as this stimulated the enzyme reaction (Fisher, Krasna & Rittenberg, 1954). The exchange rate (R_g) was calculated by the method of Farkas & Fisher (1947). The ratio of R_g value to the rate constant of the reaction measured by the methylviologen method showed a constant value of 16 with different enzyme samples from intact cells or sonic extracts of *Proteus vulgaris*, suggesting that both reactions are catalyzed by the same enzyme. The methylviologen method was satisfactory with intact cells and extracts of *Escherichia coli*, *Clostridium pasteurianum*, but not with *C. welchii*, because of the presence of an enzyme decomposing dithionite.

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Further Observations on the Specificity of Uridine-diphosphate-glucuronic Acid as Glucuronyl Donor. By G. J. DUTTON and J. H. SPENCER. (*Department of Biochemistry, Queen's College, Dundee, University of St Andrews*)

Uridine-diphosphate-glucuronic acid (UDPglucuronic acid) (Dutton & Storey, 1953; Storey & Dutton, 1955) is necessary for synthesis, in liver homogenates, of the 'ether'-linked *o*-aminophenyl- and (−)-menthyl-glucuronides (Dutton & Storey, 1951, 1954), and of the 'ester'-linked *o*- and *p*-aminobenzoylglucuronides (Dutton, 1955). Phenolphthalein, thyroxine, certain corticosteroids (Isselbacher & Axelrod, 1955) and other steroids (Storey & Dutton, 1956) have been similarly conjugated, UDPglucuronic acid again acting as glucuronyl donor.

The work reported here suggests that, in addition to these compounds, certain aliphatic alcohols and fatty acids also accept glucuronic acid from the nucleotide, which thus apparently functions as glucuronyl donor to a wide range of aglycones (ROH) whose only structural requirement would seem to be an hydroxyl group:

ROH + UDPglucuronic acid →
R-O-glucuronic acid + UDP.

The enzyme system responsible is in each case located in the small particulate, or microsomal, fraction of the homogenate (Dutton & Storey, 1954; Strominger, Kalckar, Axelrod & Maxwell, 1954).

α-Ethylhexanoic acid and *tert.*-amyl alcohol were selected from those aliphatic acids and alcohols known to be excreted as glucuronides (Kamil, Smith & Williams, 1953*a*, *b*). Presence of UDPglucuronic acid and $1{\cdot}38 \times 10^{-3}$M α-ethylhexanoic acid during incubation was necessary for formation of an ethyl acetate-extractable compound giving a positive naphthoresorcinol reaction. β-Glucuronidase treatment destroyed this compound; such destruction was strongly inhibited by boiled saccharate solution (see Levvy, 1952). Thus the compound appeared to be a glucuronide (see Dutton, 1955). Known 2-ethylhexanoylglucuronide behaved likewise.

The compound was hydrolysed with acid (Kamil, Smith & Williams, 1953*b*), the aglycone extracted with ether and chromatographed beside known α-ethylhexanoic and related acids; it travelled like known α-ethylhexanoic acid.

tert.-Amyl alcohol glucuronide formation was indicated similarly, with substrate molarity of $1{\cdot}42 \times 10^{-2}$, conjugate extraction with ethanol-ether mixture from acidified $(NH_4)_2SO_4$ solution, and aglycone chromatography (after enzymic hydrolysis of the conjugate) utilizing xanthogenate formation (Kariyone & Hashimoto, 1951). The supposedly pure glucuronide behaved comparably.

Competitive inhibition occurred when either substrate was added to the *o*-aminophenylglucuronide-synthesizing medium (Dutton & Storey, 1954), suggesting that the same enzyme system might be involved. pH and oxygen requirements were those found for other substrates, and again synthetic activity was located in the microsomes.

'Crude factor' was used in preliminary studies, confirmation being obtained with a pure preparation (Storey & Dutton, 1955).

We thank Professor R. T. Williams for gifts of substances and the Medical Research Council for a grant.

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Glucose Catabolism by Strains of *Trypanosoma rhodesiense*. By P. T. GRANT and J. D. FULTON. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The blood-stream form of the pathogenic African trypanosome, *Trypanosoma rhodesiense*, utilizes glucose at a high rate when incubated at 37° in a phosphate-buffered saline medium (pH 7·4) containing 0·2% (w/v) glucose and 10% (v/v) rat serum. The products of this catabolism have been identified as ethanol, formate, acetate, pyruvate, succinate and glycerol (Fulton & Stevens, 1945), and it has now been found that 80% of [U-^{14}C]-glucose utilized by the parasite can be accounted for as extracellular pyruvate, 0·93% being evolved as $^{14}CO_2$. The radioactivity in the respiratory CO_2

and pyruvate obtained in similar 2 hr. incubations containing either [U-^{14}C]-, [3,4-^{14}C]-, or [1-^{14}C]-glucose and the distribution of isotope in the pyruvate samples was consistent with the view that glucose is catabolized via the classical glycolytic pathway (the Embden–Meyerhof–Parnas scheme). Essentially similar results were obtained with drug-resistant (stilbamidine-fast and atoxyl-fast) strains of the same trypanosome.

Pyruvate is thus the main end-product of glucose catabolism by the parasite under *in vitro* conditions, and neither coenzyme A, diphosphopyridine nucleotide (DPN^+ or DPNH), thioctic acid, cocarboxylase and yeast extract added singly or together to the incubation medium, nor substrates such as oxaloacetate, citrate and CO_2, had any marked effect on the amounts of pyruvate and respiratory CO_2 produced. Moreover, it seems likely that pyruvate is also the main end-product of glucose catabolism of the growing trypanosome *in vivo*, since the blood-pyruvate levels of rats become enormously enhanced during the later stages of an infection.

At the end of a 2 hr. incubation period with [U-^{14}C]glucose, 1·73% of the radioactivity in the glucose utilized had been incorporated into the cellular material of the parasite. 0·9% was present as fat and was almost entirely associated with glyceride-glycerol; fatty acids contained only traces of radioactivity. In the trypanosome protein (containing 0·64% of the radioactivity of the glucose utilized), only aspartic acid, alanine, glycine and serine contained ^{14}C. For these reasons, the lack of appreciable radioactivity in fatty acids and protein-bound glutamic acid would indicate that certain reactions of the tricarboxylic acid cycle do not take place to a significant extent under *in vitro* conditions.

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The Metabolism of Ethyl Mercaptan. By G. A. Snow. (*Imperial Chemical (Pharmaceuticals) Limited, Hexagon House, Blackley, Manchester*, 9)

It has been found independently by several groups of workers (Del Pianto, 1953; Brown *et al.* 1954; Kushner *et al.* 1955; Davies *et al.* 1956) that ethyl mercaptan and related compounds exhibit a remarkable antitubercular activity in experimental animals. This specific activity occurs only *in vivo*; when tested *in vitro*, ethyl mercaptan shows only the feeble inhibitory activity common to other alkyl thiols. Interest has therefore centred on the metabolism of ethyl mercaptan.

Diethyl disulphide and ethyl thiolbenzoate labelled with ^{35}S were prepared and administered to mice and guinea pigs. Examination of the tissues showed that radioactivity was quickly and uniformly distributed throughout the body with no observable tendency to concentrate in a particular organ or tissue. Some radioactivity (10–15% of the dose) appeared in the breath, little in the faeces, but most was excreted in the urine. Urinary excretion was exponential, 50% of the total recoverable radioactivity appearing in the first 10 hr. In both mouse and guinea pig about 85% of the urinary radioactivity was due to sulphate, but the remainder was largely represented as a single radioactive spot on paper chromatography. An identical spot, but without any indication of sulphate, was given by extracts of tissues from dosed mice. From experiments with radioactive guinea-pig urine the organic metabolite appeared to be neutral, chemically stable and somewhat volatile.

Urine from guinea pigs dosed with unlabelled diethyl disulphide was extracted with chloroform, ether-soluble material was removed, and the resultant product distilled. The main fraction boiling at 60–70°/0·05 mm. was recrystallized from ether to give long needles (m.p. 33·5°) of ethyl methyl sulphone. The identity of this compound with the metabolite was checked by comparing ethyl methyl [^{35}S]sulphone with concentrates from the urine of animals dosed with $(C_2H_5{}^{35}S)_2$. Indistinguishable R_F values were observed on paper chromatograms run with three different solvents. Counter-current distribution of the urine concentrate using a chloroform–water system confirmed the sulphone as the main metabolite but also showed the presence of another which had not been revealed by paper chromatography. The constitution of the second metabolite is undetermined. Neither metabolite possesses significant antitubercular activity. The biochemical significance of the metabolism of ethyl mercaptan will be discussed.

The author thanks Dr A. R. Martin for dosing and care of animals.

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The Isolation of Further Vitamin B_{12} Analogues. By K. H. Fantes and Cynthia H. O'Callaghan. (*Glaxo Laboratories Ltd., Sefton Park, Stoke Poges, Bucks.*)

Ford, Holdsworth & Kon (1954, 1955) have shown that the vitamin B_{12}-requiring *Escherichia coli* mutant was capable of producing vitamin B_{12} analogues from factor B and certain benziminazoles or purines. We isolated an analogue from the metabolite fluid after a fermentation by *S. griseus* in the presence of *o*-phenylenediamine, but without factor B (Fantes & O'Callaghan, 1954, 1955). Further analogues were prepared by this method.

The addition of graded levels of 1:2-diamino-4:5-dichlorobenzene did not lead to an appreciable production of the 5:6-dichlorobenziminazole analogue, though this was formed readily when 5:6-dichlorobenziminazole itself was used. 20 μg./ml. of the latter completely suppressed the production of vitamin B_{12} so that only the new analogue was formed, whereas mainly vitamin B_{12} was formed in the presence of the diamine (even at 80 μg./ml.). The chlorine-containing analogue was isolated by methods previously described (Fantes & O'Callaghan, 1955). In a similar fashion crystalline vitamin B_{12} analogues containing 2:3-naphthiminazole and 5-aminobenziminazole were isolated after fermentations in presence of 80 and 250 μg./ml. of the respective precursors. The addition of 0·05% sodium hydrosulphite to the medium helped to produce the aminobenziminazole compound. From the same fermentation broth another crystalline factor was obtained, probably the 5-hydroxybenziminazole analogue (B_{12}-Factor III, Friedrich & Bernhauer, 1955), since it appeared to be identical with the product obtained from nitrous acid treatment of the 5-aminobenziminazole analogue.

5:6-Dichlorobenziminazole, 2:3-naphthiminazole and 5-aminobenziminazole were all identified in the acid hydrolysates of the respective B_{12} factors by their R_F values and u.v. absorptions; the aminobenziminazole also gave a pink colour on coupling with β-naphthol. The constitution of the chlorine-containing analogue was confirmed by X-ray crystallographic evidence (Kamper & Hodgkin, 1955).

A number of other micro-organisms have been found to be able to form analogues, but most of them seem unable to incorporate benziminazoles substituted in positions 2 or 4, or purines. Certain Clostridia, such as *C. tetani* and *C. welchii*, are able to form analogues containing purines, 4-substituted benziminazoles or triazoles.

The two analogues so far tested (containing 5:6-dichlorobenziminazole and 2:3-naphthiminazole) have been found active in the treatment of pernicious anaemia patients. The clinical trials were kindly carried out by Professor G. M. Wilson, Department of Pharmacology and Therapeutics, at the University of Sheffield.

Other biological tests are in progress.

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Chromogenic and Fluorogenic Reactions of Steroids in Concentrated Acids. By H. Kalant* (introduced by F. G. Young). (*Department of Pathological Chemistry, University of Toronto, Canada*)

Treatment with concentrated sulphuric, phosphoric or other acids has been used for identification and measurement of many steroids, by absorption spectrophotometry or fluorophotometry of the reaction mixtures. Attempts to correlate reactivity with the number of hydroxyl groups or other substituents in the steroids (Goldzieher, Bodenchuk & Nolan, 1954; Linford & Paulson, 1952) have yielded no satisfactory explanation of the mode of action of concentrated acids on steroids.

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In the present work, absorption spectra of various adrenocortical, androgenic, oestrogenic, and other steroids were compared after treatment with concentrated H_2SO_4 (Zaffaroni, 1953) and with 99–100% H_3PO_4 (Kalant, 1954). Agreement between the two spectra varied from excellent with hydrocortisone to almost none with oestriol. Spectra in H_3PO_4 were stable for at least 24 hr. Those in H_2SO_4 underwent changes with time, or on heating, which increased considerably their resemblance to the H_3PO_4 spectra. Progressive dilution of both sets of reaction mixtures produced striking sequences of change in absorption patterns, which were essentially similar in both acids for a given steroid. In general, corresponding spectra were found at lower concentrations of H_2SO_4 than of H_3PO_4.

Examination of the reaction mixtures at each stage of dilution, under different visible and ultraviolet illuminations, showed the same changes in visible fluorescence in both sets. Each steroid behaved in a different manner. For example, cortisone in concentrated acid showed blue fluorescence under 365 mμ. illumination, which decreased progressively on dilution. In contrast, hydrocortisone in concentrated acid showed pale blue-white fluorescence under 365 mμ. and bright yellow-green under 436 mμ.; on dilution, fluorescence under 436 mμ. disappeared, but a brilliant green appeared under 365 mμ. Examination of the fluorescences by spectrofluorometry (Gornall & Kalant, 1955) and by spectrography showed each to be a complex emission of two or more bands which altered in relative intensity on dilution.

Ethylene chloride extracts of H_2SO_4 reaction mixtures, after either neutralization or extreme dilution, were chromatographed on paper. Individual fractions were eluted, redissolved in concentrated acid, and their absorption and fluorescence properties studied, including the changes on redilution.

All the findings suggest a general reaction pattern involving formation of an unstable intermediate which undergoes degradation into several different stable products, capable of reversible associations in acid. No evidence was found for the polymerization hypothesis of Jaffe (1952).

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Carbamyl Phosphate-Aspartate Transcarbamylase. By J. M. Lowenstein* and P. P. Cohen.
(*Department of Physiological Chemistry, University of Wisconsin, Madison, U.S.A.*)

It has been shown previously that carbamyl aspartate (CA) is formed enzymically from aspartate and an intermediate compound which acts as carbamyl group donor (Lowenstein & Cohen, 1954, 1955). Similar conclusions were reached by Reichard & Hanshoff (1955). The identification and synthesis of carbamyl phosphate (Jones, Spector & Lipmann, 1955) has greatly facilitated the study of CA biosynthesis.

The enzyme ('carbamyl phosphate-aspartate transcarbamylase') which forms CA requires only L-aspartate and carbamyl phosphate. CA formation has been assayed by a method which makes use of the fact that the ^{14}C-carbamyl carbon of carbamyl phosphate, but not that of CA, is expelled as $^{14}CO_2$ when heated with dilute HCl. The enzyme occurs predominantly in the supernatant obtained after high speed centrifugation of rat-liver homogenates. It has an optimum pH of 9·2, and is inhibited 50% by 5×10^{-3}M iodoacetate, 70% by 1×10^{-3}M *p*-chloromercuribenzoate, and 70% by 1×10^{-1}M arsenate. The enzyme has been found to be present in the following rat tissues: brain, gastric mucosa, intestinal mucosa, kidney, liver, lung, leg muscle, pancreas, spleen and testis. Its presence in rat blood and heart is doubtful. It occurs also in pigeon tissues, a finding which suggests that carbamyl phosphate is synthesized in birds. The specificity of the carbamyl phosphate transcarbamylase reaction has been studied in supernatants obtained by low speed centrifugation of rat-liver homogenates. Out of thirty amino acids tested, only ornithine and aspartate react readily as acceptors of the carbamyl group. Very low rates of reaction are also observed with asparagine, *iso*-asparagine and lysine.

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Species Differences in the *o*- and *p*-Hydroxylation of Aniline. By D. V. PARKE and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

Some carcinogenic aromatic amines appear to produce their biological effects after conversion *in vivo* to *o*-aminophenols. In the case of 2-naphthylamine there appears to be a correlation between the amounts of 2-amino-1-naphthol conjugates in the urine and the incidence of bladder tumours in different species, and this provides a possible explanation of the differing susceptibilities of various species to the action of the carcinogen (Bonser, Clayson & Jull, 1951).

During an investigation of the metabolism of ^{14}C-aniline it became possible, by the isotope dilution technique, to measure the extent of *o*- and *p*-hydroxylation in various species. The urine of animals given 250 mg./kg. of aniline was collected for 48 hr. and the total amounts of *o*- and *p*-aminophenol excreted were determined. The average *para*/*ortho* ratio for two animals of each of the species so far investigated were: gerbil, 15; guinea pig, 11; hamster, 10; rabbit, 6; rat, 5; mouse, 3; ferret, 1; cat, 0·4. The total amino phenols excreted in this time was 40–60% of the dose. It is to be noted that the ferret and cat produce much more *o*-isomer than the others.

One possible explanation of these results is that two enzymes are involved, an '*ortho*-hydroxylase' and a '*para*-hydroxylase', and that these enzymes occur in different proportions in different species. This possibility is now being investigated. Another possibility is that some animals can acetylate the aromatic amino group more extensively than others and consequently *ortho*-hydroxylation is sterically hindered. However, it is known that the cat can acetylate aromatic amines and therefore this explanation seems unlikely. Other animals (including the dog which does not acetylate aromatic amines, cf. Marshall, 1954) are being studied.

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DEMONSTRATIONS

Total Body Water in Obesity. By G. L. S. PAWAN. (*Professorial Medical Unit, The Middlesex Hospital, London, W.* 1)

Total body water was estimated by the urea method of McCance & Widdowson (1951) with precautions described by Pawan (1954).

In 18 apparently normal non-obese subjects (10 males, 8 females, aged 18–54 years), the urea space ranged from 58 to 66 (mean 62) % of the body weight. In a series of 34 obese patients (4 males, 30 females, aged 16–66 years), 28–320 (mean 63) % above ideal body weight for age, sex and height, the urea space was found to be 25–58 (mean 46) % of body weight. Total body water expressed as a percentage of body weight was found to vary inversely with the degree of adiposity.

When weight reduction (mean 10·2 kg.) was induced in the obese series of patients by calorie restriction, the mean absolute value for total body water decreased by 1·9 l., but expressed as a percentage of body weight, the value increased from 46 to 49% of body weight.

Reasonably good agreement was found between the urea, antipyrine (Soberman *et al.* 1949), and *N*-acetyl-4-amino-antipyrine (NAAP) (Brodie *et al.* 1951) spaces. In 7 obese subjects, the mean value for the antipyrine space was 4·9% less than the mean NAAP space. The mean urea space was 2·8% greater than the antipyrine space.

I thank Professor Alan Kekwick for permission to investigate the patients reported.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **350th Meeting** *of the Biochemical Society was held in the Department of Biochemistry of the University of Glasgow on 13th April* 1956, *at* 11.30 *a.m., when the following papers were read:*

COMMUNICATIONS

The Effect of Iron Status on the Pattern of Free Amino Acids and Organic Acids in Plant Leaves.

By P. C. DE KOCK and R. I. MORRISON. (*The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen*)

The amino and organic acids of leaves of plants of differing iron status have been studied by chromatographic methods. The leaves were macerated in a Waring Blendor in 80% acetone (HCl acidified), and the extract passed over Zeo-Karb 225 and IR-4b resins after evaporation of the acetone and other procedures. The amino acids in the eluate from the Zeo-Karb were studied semi-quantitatively by 2-dimensional paper chromatography (phenol–ammonia and butanol–acetic acid–water). Organic acids were quantitatively estimated on a silica gel column using gradient elution with *tert.*-amyl alcohol–chloroform mixtures. Inorganic analyses for iron, phosphorus, calcium and potassium were conducted by methods current at this institute.

All iron-deficient plants were found to contain larger amounts of free amino acids in both roots and leaves than did healthy green plants. Iron-toxic leaves on the other hand, even when showing chlorosis, had smaller quantities of free amino acids than the healthy leaves.

The total extractable organic acids were found to increase with increasing iron status, thus showing an inverse relationship to the amino acids. Also, iron-deficient plants were seen to contain relatively large amounts of the tricarboxylic acids whereas iron-toxic plants contained more of the dicarboxylic acids (malic, malonic). From the result it appears that the Krebs cycle is operative in plants and that the relative amounts of acids present in the leaves is dependent on the iron status, as measured by the phosphorus-iron ratio. It was thus found that mustard plants poisoned with fluoroacetic acid, an inhibitor of aconitase, showed symptoms of typical iron deficiency with high phosphorus-iron ratio, increased amounts of free amino acids and altered organic acid pattern.

Faecal Excretion in Man after the Administration of Mixed Plant Sterols (Sitosterols).

By SHEENAH K. BISSET and R. P. COOK. (*Department of Biochemistry, Queen's College, Dundee* (*University of St Andrews*))

The observations of Peterson and his colleagues (e.g. Peterson, Nichols & Schneour, 1952) that sitosterols hinder the absorption of cholesterol in experimental animals has been extended by various workers to the effect on cholesterol absorption in man (e.g. Pollak, 1953; Joyner & Kuo, 1955). It has been shown that in the blood plasma the level of cholesterol and of lipoproteins is lowered. The experiments to be described were made in an attempt to ascertain the mode of action of vegetable sterols.

Three subjects were fed 10 g. per day of mixed sitosterols for 5 days and the faeces collected before, during and after administration. An ethereal extract was analysed for total lipid and for free and total sterol. The weight of faeces and the amount of lipid (30–40% of the dry wt.) were increased after sitosterol administration. Total sterol increased in amount, but on balance was equal to only about 60% of that fed. There was an increase in the ester sterol excreted, values of from 1 to 1·5 g. per day being found.

The lipids were separated by chromatography on alumina and a fraction rich in sitosteryl esters obtained. It is possible that one mechanism of action of the sitosterol is that it competitively inhibits the esterification of cholesterol in the gut. There is evidence also that 'mixed' crystals of the free sterols are formed. The conversion of sitosterol to non-digitonin precipitable material is still unexplained.

The feeding experiments and collection of faeces were carried out under the supervision of Drs M. F. Oliver and G. S. Boyd, Edinburgh. We are grateful to Dr Max Tishler, Merck and Co. Inc. for a generous gift of sitosterols. One of us (S.K.B.) was in receipt of a personal grant from the Scottish Hospitals Research Trust.

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The Hydrolysis of Glucose 1-phosphate by Rat Liver Extracts. By G. A. J. GOODLAD* and G. T. MILLS. (*Biochemistry Department, University of Glasgow*)

It was suggested by Broh-Kahn & Mirsky (1948) that no specific glucose 1-phosphatase activity exists in liver and that glucose 1-phosphate (G 1-P) is converted to glucose 6-phosphate (G 6-P) before hydrolysis. Swanson (1950) partially purified a specific glucose 6-phosphatase from liver which had no activity towards G 1-P. Faulkner (1955), on the other hand, has demonstrated the presence in silkworm blood of a phosphatase specific towards G 1-P. As part of a study of the specificity of rat liver phosphomonoesterases we have examined the mechanism of hydrolysis of G 1-P by rat liver extracts.

The hydrolysis of G 1-P by freshly prepared liver homogenates occurred at a higher pH optimum and was more strongly activated by magnesium ions than the hydrolysis of G 6-P. Substrate inhibition was absent when G 6-P was being hydrolysed but was very pronounced with G 1-P as substrate. Rat liver phosphoglucomutase was found to have a pH optimum similar to that for the hydrolysis of G 1-P, to require magnesium ions for activity and to be inhibited by high concentrations of G 1-P. These findings are in support of the views of Broh-Kahn & Mirsky as they are most readily explained by G 1-P undergoing an initial rate controlling mutation to G 6-P.

The intracellular distribution of glucose 1-phosphatase activity was studied. When the different fractions, obtained by differential centrifugation, were assayed singly, negligible activities were obtained. On assaying combined microsomal and non-particulate fractions, however, 85% of the activity of the unfractionated cytoplasm was obtained. Boiled microsomal fraction added to unboiled non-particulate fraction and vice versa failed to show this effect. These results again indicate that the hydrolysis of G 1-P is a two-stage process involving phosphoglucomutase, which occurs only in the non-particulate fraction (Hers, Berthet, Berthet & de Duve, 1951) and glucose 6-phosphatase which was found to be present mainly in the microsomes and to only a very small extent in the non-particulate fraction in these preparations.

No evidence in support of any secondary mechanism for the hydrolysis of G 1-P was obtained in these studies. At pH 5·5, the optimal pH of Faulkner's glucose 1-phosphatase, the rate of hydrolysis by liver homogenates was negligible.

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The Multiple Nature of Rat Liver Acid Phosphatase Activity. By G. A. J. GOODLAD* and G. T. MILLS. (*Biochemistry Department, University of Glasgow*)

Studies of the effect of magnesium ions on the pH-activity curves of rat liver acid phosphatase activity using various substrates have indicated the existence of a Mg^{2+}-sensitive enzyme (I), optimally active around pH 3·75, and a Mg^{2+}-insensitive enzyme (II), optimally active at pH 5·5. When β-glycerophosphate and adenosine 3′-phosphate were used as substrate, evidence was obtained for the presence of a third enzyme optimally active about pH 6·5.

The shape of pH-activity curves obtained using veronal-acetate buffers differed from those obtained using acetate buffers. Veronal appeared to activate I and inhibit II. Copper ions had no effect on I but inhibited II completely, even at concentrations of 2×10^{-4}M. I was inhibited by citrate and tartrate to a much greater extent than was II.

A partial separation of I and II was achieved by fractionation with acetone in the presence of zinc ions followed by electrophoresis in the Tiselius apparatus.

No difference in the intracellular distribution of the three enzymes was found.

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Cytidine Diphosphate Glycerol and Related Compounds from *Lactobacillus arabinosus*. By J. Baddiley, J. G. Buchanan, B. Carss, A. P. Mathias and A. R. Sanderson. (*Department of Chemistry, University of Durham, King's College, Newcastle upon Tyne*)

By the use of gradient elution chromatography on Dowex-2 with formic acid, followed by charcoal adsorption and elution, it has been possible to obtain pure samples of the two cytidine nucleotides previously isolated from *Lactobacillus arabinosus* (Baddiley & Mathias, 1954). One of these has now been identified as cytidine diphosphate glycerol (CDP-glycerol). Analysis indicates the presence of two phosphate groups to one cytidine residue. On hydrolysis in N-hydrochloric acid at 100° for 20 min. cytidine 5′-phosphate and two organic phosphates are produced. These organic phosphates have the same R_F values in several solvents as a mixture of α- and β-glyceryl phosphates, the α-form being present in greater amount. The glyceryl phosphates are readily separated on paper, using a *tert.*-butanol–picric acid–water solvent (Hanes & Isherwood, 1949). Only the α-form gave a reaction typical for a 1:2-diol when the paper was sprayed with periodate followed by Schiff's reagent (Buchanan, Dekker & Long, 1950). Confirmation of the identity of the α-phosphate was obtained by oxidation with periodate to glycollic aldehyde phosphate which had the same R_F as the periodate product from authentic α-glyceryl phosphate. The mixture of α- and β-phosphates was dephosphorylated enzymically (polidase) giving glycerol and orthophosphate.

When the nucleotide was treated with phosphatases obtained from the venom of *Crotalus atrox* the products were cytidine, inorganic phosphate and α-glyceryl phosphate. It was shown by paper chromatography that no β-glyceryl phosphate was present. This indicates that the original nucleotide contained an α-glyceryl phosphoryl residue.

The presence of a pyrophosphate linkage between the 5′-position in the cytidine and the α-position in the glycerol, already strongly suggested from the above evidence, was confirmed by alkaline hydrolysis. When heated for 20 min. in dilute ammonia the original substance was partly converted into cytidine 5′-phosphate and glyceryl-1:2 hydrogen phosphate. The identity of this cyclic phosphate was established by comparison on paper chromatography and electrophoresis with an authentic sample.

The second cytidine nucleotide is also a derivative of cytidine 5′-pyrophosphate bearing a substituent on the terminal phosphate group. The nature of this substituent has not yet been fully established, but from the reactions of this nucleotide it appears to be closely related to CDP-glycerol.

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Studies on the Reduction of Nitrate in the Artificial Rumen in the Presence of Various Substrates. By A. J. G. Barnett and I. B. R. Bowman. (*Division of Agricultural Biochemistry, Department of Biological Chemistry, University of Aberdeen*)

The condition of 'oat-hay' poisoning in cattle has been investigated by a number of workers, including Bradley, Eppson & Beath (1939), and is related to high concentrations of nitrate in the feed. These may reach a level of 7% (as KNO_3 on the D.M.) in oat straw (Sjollema, 1931). Nitrate is reduced, by microbial action, to nitrite in the rumen with a resultant toxic effect manifested partly by the occurrence of methaemoglobinuria.

Studies of the reactions involved have been made by Sapiro, Hoflund, Clark & Quin (1949) using rumen liquor, which was incubated without regard to sustaining pH at normal rumen levels, and by Lewis (1951*a*) who performed his experiments on a sheep which had not been fed for 16 hr. This latter worker (1951*b*) established, from *in vitro* studies, that various substances normally present in crops could act as hydrogen donors in the reduction.

In the present work, six artificial rumens (Burroughs, Headley, Bethke & Gerlaugh, 1950) were set up, each containing a rumen liquor inoculum and a mineral salt medium. Two rumens acted as controls, to the third was added cellulose, to the fourth, glucose, to the fifth, dried grass and to the sixth, water-soluble grass pigments. CO_2 was passed through the rumens individually, and they were kept at 39° for 35 hr.; 5 hr. after the start of the run, equal volumes of a solution of KNO_3 were added to each. The emergent gases

were tested for ammonia and nitric oxide but neither were detected, probably as a result of controlling the pH of the medium at pH 6·5–6·7. Nitrate, nitrite, ammonia and protein N were determined on samples collected at intervals, and it was found that nitrite formation was most vigorous when dried grass or grass pigments (containing potential hydrogen donors) were present. Cellulose decreased the apparent nitrite production beneath that attained in the controls but no nitrite was detectable in the rumen containing glucose although the nitrate disappeared. Small amounts of ammonia were produced in all cases while the total recovery of NO_3-N, NO_2-N and NH_3-N was good in all cases. The presence of nitrate and nitrite did not appear to have a completely inhibitory effect on protein synthesis.

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Yeast Branching Enzyme. By D. J. Manners and Khin Maung. (*Department of Chemistry, University of Edinburgh*)

During the purification of phosphorylase and isoamylase from brewer's yeast (Manners & Khin Maung, 1955) we observed that certain protein fractions catalysed a rapid decrease in the iodine staining power of amylose. Further investigation has shown that this activity was caused by a branching enzyme, i.e. an amylose → glycogen transglucosidase.

By ethanol-citrate fractionation (at −5°) of a brewer's yeast extract, we have obtained a protein preparation which reduced the iodine staining power of amylose, soluble starch, amylopectin, and amylopectin β-dextrin without concomitant liberation of reducing groups. On incubation of amylose (B.V. 1·33; β-amylolysis limit 84%) with branching enzyme, the iodine absorption value at 680 mμ. decreased by 66 and 81% within 1 and 4 hr. respectively; the β-amylolysis limit likewise decreased, to 50 and 48%. After 20 hr. incubation, the end-product stained red-brown with iodine, the absorption spectrum having a maximum at 520 mμ. (cf. amylopectin $\lambda_{max.}$ 540 mμ., glycogen 460–490 mμ.). Yeast branching enzyme has no action on glycogen or glycogen β-dextrin.

Enzyme action is inhibited by mercuric chloride (5×10^{-4}M); it is optimum at pH 7·0 and 20°, these conditions being similar to those for the branching enzymes (*Q*-enzymes) of plants (Hobson, Whelan & Peat, 1950). Unlike the branching enzyme from *Polytomella coeca* (Barker, Bebbington & Bourne, 1953), the yeast enzyme is not activated by maltosaccharides.

The branch points synthesized by yeast branching enzyme are hydrolysed by yeast isoamylase, and are therefore similar to those in amylopectin and glycogen (i.e. α-1:6-glucosidic). Treatment of amylose with branching enzyme for 4·5 hr. gave a branched polysaccharide with a β-amylolysis limit of 45%. After treatment with isoamylase, the β-amylolysis limit was 65% (cf. Manners & Khin Maung, 1955).

We are grateful to Professor E. L. Hirst, F.R.S., for his advice and encouragement.

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The Metabolism of Chick Embryonic Heart and Liver Tissue During Adaptation to Tissue Culture Conditions. By J. Paul and Enid S. Pearson. (*H.E.R.T. Tissue Culture Laboratory, Department of Biochemistry, University of Glasgow*)

Respiration, ^{32}P incorporation, protein, lipid, carbohydrate, RNA and DNA content and the metabolism of glucose, lactate and pyruvate have been studied in fresh 15-day chick embryonic heart and liver and during the 6 days following explantation and maintenance as tissue cultures.

Fresh heart tissue initially has a very low Q_{O_2} under these conditions. After 24 hr., respiration is significantly greater and is depressed by renewal of the medium. Heart explants utilize glucose rapidly, producing large amounts of lactate. Pyruvate accumulates in the medium to a maxi-

mum concentration and may fall later if the medium is not renewed. ^{32}P incorporation into lipid phosphorus, acid-soluble phosphorus, RNA phosphorus and DNA phosphorus decreases rapidly in the first 24 hr. On renewal of the medium incorporation is stimulated and it may exceed the initial level. Nucleic acids in the tissue rapidly decrease in the first two days. Tissue carbohydrate also decreases while other constituents show little change. Histological sections show rapid disappearance of muscle cell nuclei in the centre of the explant, with better survival at the periphery. Fibroblasts proliferate and form a capsule round the explant as well as growing over the surface of the culture vessel.

Fresh liver tissue has a high Q_{O_2}. Lactate disappears from the medium. Glucose also disappears, but the total amount of hexose in the medium increases at first due to the appearance of non-reducing material. After 24 hr. lactate utilization has ceased and it has accumulated to a level higher than that originally present. It continues to accumulate. Respiration has fallen to a low level. It is not stimulated by renewing the medium. As in heart, there is a marked fall in nucleic acids without much alteration in other tissue constituents. The pattern of ^{32}P incorporation is similar to that observed in heart, the fall in activity in the first day being very pronounced. It is stimulated by renewing the medium. Histological sections show a course of events similar to those in heart explants but the necrotic changes are greater.

We are indebted to Professor J. N. Davidson for the provision of facilities, to Miss Ina Withers for technical assistance and to the Scottish Hospitals Endowment Research Trust for financial support in this research.

The Excretion of 5-Hydroxyindoleacetic Acid (5-HIAA) by Normal Human Subjects. By M. Dick and A. Todrick. (*Department of Clinical Research, Crichton Royal, Dumfries*)

The isolation of 5-hydroxytryptamine (5-HT) from mammalian plasma and intestinal mucosa, and its subsequent identification in the human body, has aroused considerable speculation as to its physiological function. Erspamer (1954) holds that urinary 5-HIAA excretion is the best indication of the level of 5-HT metabolism.

The presence of 5-HT has been demonstrated in the central nervous system (Amin, Crawford & Gaddum, 1952), and the observation of Gaddum (1953) that the hallucinogenic drug lysergic acid diethylamide is a powerful inhibitor of the action of 5-HT on smooth muscle indirectly supports the view that 5-HT may influence central nervous function (cf. Pletscher, Shore & Brodie, 1955; Rodnight & McIlwain, 1956).

Despite the interest in 5-HIAA the published data on its 24 hr. urinary excretion in humans is limited.

The method employed for the estimation was a modification of that of Udenfriend, Titus & Weissbach (1955). 5-HIAA was estimated in the 24 hr. urines of twenty males and twenty-one females. The males gave a mean of $4{\cdot}20 \pm 1{\cdot}20$ and the females $3{\cdot}72 \pm 0{\cdot}99$ (S.D.) mg./day. A 't' test indicates that these means are not significantly different ($P = 0{\cdot}2$). The combined results have a mean of $3{\cdot}95 \pm 1{\cdot}11$ mg./day. The observed range is 2·2–7·8, the distribution curve being slightly skewed. These results agree with those of Udenfriend *et al.* (1955), four cases, range 2–8 mg./day; Erspamer (1955), five cases, mean 4·5 mg./day and Snow, Lennard-Jones, Curzon & Stacey (1955) forty cases, mean 2·7 μg./ml. urine.

There does not appear to be any correlation of 5-HIAA excretion with age or weight.

When the results are plotted against urine volume, there is some suggestion of a direct correlation. However, in all cases where individual volumes varied widely on successive days, no concomitant variation in 5-HIAA excretion was noted.

The day-to-day variation in 5-HIAA excretion over a period of about 1 week appears to be fairly small, normally not varying by more than 1 mg./day.

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Changes in Glucose Utilization with the Growth of Tissue Cultures of HeLa Cells. By W. C. FULTON, R. SINCLAIR and I. LESLIE. (*Biochemistry Department, Queen's University, Belfast, Northern Ireland*)

HeLa Cells (a strain of human epidermoid carcinoma) were grown in roller tubes with daily changes of a medium consisting of 5% chick embryo extract, and 20% human serum in modified Connaught medium 858 (Healy, Fisher & Parker, 1955). The glucose (G) utilization, lactic acid (LA) and total keto-acid (KA) production were measured over consecutive periods of 2 days and compared with changes in phospholipid, ribonucleic acid and deoxyribonucleic acid phosphorus (DNA-P). Since the average amount of DNA-P per nucleus of the HeLa cell is constant at 1·36 pg.* (Leslie, Fulton & Sinclair, 1955), the cell number can be estimated from DNA-P determinations.

Previous attempts to correlate glucose utilization with increases in cell material or cell number indicated that there was no direct relationship between these two quantities (Willmer, 1942; Westfall, Evans, Shannon & Earle, 1953). Similarly, with the HeLa cells, the glucose utilization did not run parallel to increases in cell number, since, for example, it increased only by about 10% for a fourfold increase in cell number.

Measurements of lactic acid and total keto-acid production revealed that the proportion of glucose appearing as these compounds diminished with increasing cell number. Presumably, as the glucose concentration of the medium falls, an increasing proportion of the lactic and keto-acid is used for energy production. In support of this view, it was found that there was a linear relationship between the cell number and the glucose utilized but not accounted for as lactic and keto-acid.

While lactic acid production could account for nearly half the glucose utilized (LA/G molar ratio approx. 0·9) in the initial cultures, the total keto-acid represented less than 5% (KA/G molar ratio = 0·05 or less). With increasing cell number, the ratio of lactic acid to keto-acid (LA/KA) tends to fall. Insulin has been found to stimulate the growth of HeLa cell cultures (Leslie *et al.* 1955), but in this case the ratio LA/KA in the medium always rises significantly. From these results it can be concluded that the change in LA/KA under insulin treatment is not simply a consequence of the additional growth.

The authors thank Dr G. W. Sheffield, Department of Bacteriology, University College Hospital Medical School, London, for the cultures of HeLa cells which enabled us to build up our stock.

* pg. = picogram = 1×10^{-12} g.

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The Influence of the Medium on the Insulin Response of Two Unrelated Human Cells Grown in Tissue Culture. By I. LESLIE, W. C. FULTON and R. SINCLAIR. (*Biochemistry Department, Queen's University, Belfast, Northern Ireland*)

Human skin fibroblasts from a 20-week embryo and from a strain of HeLa cells have been grown in roller tubes under closely comparable conditions. Two types of media have been used, a maintenance medium (MM) consisting of 20% human serum in a modified Connaught medium 858 (Healy, Fisher & Parker, 1955) and a growth-promoting medium (GPM) incorporating 5% of chick embryo extract in the maintenance medium. The insulin concentration used in all tests was (with one exception) 0·1 unit/ml.

In GPM the fibroblasts increased their number four to fivefold in 3 days, and their growth rate was uninfluenced by the presence of insulin. (Shih-man Chang (1955) found that cultures of human conjunctival cells showed a similar lack of response to insulin in growth-promoting medium.) However, insulin did act upon the fibroblasts by raising the ribonucleic acid content per cell and causing a lower lactic acid (LA) production per unit of glucose (G) utilized (i.e. a lower LA/G ratio).

Although the cell number of the fibroblasts increased by only 14% when kept in MM for 3 days, insulin produced a rise of 32% under the same conditions. From concurrent changes in cell and medium composition, it is clear that the response to insulin is greater when embryo extract is omitted from the medium, as reported by Chen (1954).

The HeLa cells were previously shown to respond to insulin by increasing their cell number and glucose uptake in growth-promoting medium (Leslie, Fulton & Sinclair, 1955). It is now found that the normal deoxyribonucleic acid content of

1·36 pg.*/nucleus is unaltered by the action of insulin (1 unit/ml.). When the HeLa cells were kept on MM, the effect of insulin was to increase the cell material rather than cell number. Glucose utilization and lactic acid production were greatly stimulated, but with this cell strain they kept in step and the LA/G ratio remained at its control value.

Both cell types respond to insulin by increased synthetic activity, but the HeLa cells obtained their energy solely by increased utilization of glucose, while the skin fibroblasts apparently oxidized a higher proportion of the glucose when insulin was present.

* pg. = picogram = 1×10^{-12} g.

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The *in vitro* Demonstration of Differential Corticosteroid Production within the Ox Adrenal Gland. By P. J. Ayres, R. P. Gould, Sylvia A. Simpson and J. F. Tait. (*Middlesex Hospital Medical School, London, W.* 1)

Indirect evidence obtained from the analysis of adrenal venous blood and histological examination of adrenal glands under various conditions has indicated that aldosterone is preferentially produced by the zona glomerulosa and cortisol by the zona fasciculata (Farrel, Banks & Koletsky, 1956). We have obtained direct evidence which supports this view. However, our results indicate that this differential production, at least when expressed as μg./wet weight tissue, may not apply to corticosterone in the ox gland.

Selected slices (0·3–0·35 mm. thick) from the ox adrenal cortex which, from histological evidence, were likely to consist mainly of either zona glomerulosa or fasciculata tissue, were incubated in separate Warburg vessels for 2 hr. under oxygen at 37° in 3·5 ml. Krebs–Ringer phosphate medium (Krebs, 1933) containing 13 mM glucose, 3 mM ATP and 3 mM fumarate. Eighteen slices from each zone, of approximately 200 mg. wet weight, were incubated. After chloroform extraction, 75% of the products was pooled for each type of tissue. Aldosterone, cortisol and corticosterone were analysed by an isotope dilution method, which included the extensive purification of the acetylated and/or free compounds (Ayres, Simpson & Tait, 1956*a*, *b*). The following mean values, with their estimated experimental errors, were obtained expressed as μg. steroid per g. wet tissue for zona glomerulosa and fasciculata tissue respectively: cortisol, $6{\cdot}5 \pm 0{\cdot}6$ and $16{\cdot}5 \pm 1{\cdot}5$; aldosterone, $4{\cdot}3 \pm 0{\cdot}6$ and $0{\cdot}7_2 \pm 0{\cdot}1$; corticosterone, $6{\cdot}1 \pm 0{\cdot}6$ and $4{\cdot}3 \pm 0{\cdot}4$. Aldosterone was further estimated and identified by various microtechniques including bioassay (Simpson & Tait, 1952) of the purified free compound. The other steroids were also further characterized.

25% of the products from the individual vessels were analysed separately. Student's t test showed the mean value for cortisol to be significantly higher and that for aldosterone lower ($P < 0{\cdot}001$) from the zona fasciculata compared with the zona glomerulosa. No individual estimations of corticosterone were made.

Some incubated crude capsule strippings of the gland gave aldosterone/cortisol ratios greater than 10. Histological examination showed that these usually consisted of capsule plus adhering zona glomerulosa tissue, but minimal zona fasciculata contamination.

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The Estimation of β-*N*-Acetylglucosaminidase. By Doreen Pugh, D. H. Leaback and P. G. Walker. (*Department of Biochemistry, Institute of Orthopaedics, Stanmore, Middlesex*)

The occurrence of β-*N*-acetylglucosaminidase in rat tissues has been demonstrated by a modification of the method of East, Madinaveitia & Todd (1941) in which liberation of phenol by the enzymic hydrolysis of phenyl *N*-acetyl-β-D-glucosaminide is used as a measure of the enzyme activity.

A stable, reproducible, partially purified enzyme preparation was obtained by fractionation of extracts of rat kidney homogenate buffered with citrate (0·1 M, pH 4·6). The precipitate obtained between 25 and 50% saturation with ammonium sulphate, after resuspension and dialysis, contained

about 80% of the total enzyme activity of the tissue and this preparation was used to study the properties of the enzyme. The pH optimum in 0·05M citrate buffer was 4·3. The Michaelis constant (K_m) for phenyl *N*-acetyl-β-D-glucosaminide was 0·002M. With increasing substrate concentration the rate of hydrolysis increased until the reaction mixture was saturated with substrate (approx. 0·03M). The amount of hydrolysis was proportional to the time of incubation and to the enzyme concentration. The amounts of phenol and *N*-acetylglucosamine liberated by the enzyme were equivalent.

In acetate buffer (used by East *et al.* 1941) activity was much lower than in citrate and inhibition produced by acetate was shown to be competitive (K_i acetate 0·018M). The hydrolysis of phenyl *N*-acetyl-β-D-glucosaminide was also inhibited by *N*-acetylglucosamine (Watanabe, 1936), acetamide and the competing substrate ethyl *N*-acetyl-β-D-glucosaminide.

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Fatty Acid Metabolism in *Sarcina lutea*. By D. MURRAY and E. A. DAWES. (*Department of Biochemistry, University of Glasgow*)

The metabolism of fatty acids by the aerobe *Sarcina lutea* has been investigated. The remarkably high endogenous respiration of freshly harvested cells was reduced by vigorous aeration for 4·5 hr. (Holms & Dawes, 1955). Substrates investigated included acetate, butyrate, valerate, caproate, heptoate, caprylate, pelargonate and caprate. All were oxidized: the total oxygen uptake, less the endogenous value, corresponded to *ca.* 0·5–0·75 oxidation of added substrate, and oxidative assimilation was indicated. Fatty acid oxidation by freeze-dried cells was also studied and no appreciable difference in the rate of oxidation by these preparations was found; freeze-dried cells were used routinely thereafter.

Protoplasts of the organism have been prepared by lysozyme treatment (Weibull, 1953*a*). Neither the rate nor the total oxygen uptake of such preparations differed from those of freeze-dried cells, with caprylate as substrate. However, cell-free preparations obtained by the action of lysozyme in hypotonic media gave a very small oxygen uptake in the presence of fatty acids. Cell-free extracts were also prepared by the method of Lamanna & Mallette (1954), but no oxygen consumption was demonstrable with fatty acids using either the whole disintegrate or the supernatant from centrifugation.

Identification of the individual enzymes involved in fatty acid degradation is under investigation. Aceto-coenzyme A (CoA) kinase was present in the supernatant of Lamanna & Mallette (1954) extracts and its dependence on CoA and adenosine triphosphate (ATP) was established. Butyrate, valerate, caproate, heptoate and caprylate also reacted. This enzyme appears to be absent from the supernatant of lysed preparations. Butyryl-CoA dehydrogenase was demonstrated in the former extracts, and since the substrate used was butyrate itself, the presence of CoA and ATP was mandatory. Of the acids tested to date, there was slight evidence for acyl-CoA dehydrogenase activity with caprylate, caprate and laurate.

Studies with preparations obtained by lysozyme treatment of *S. lutea* have shown the cytochrome pigments to be associated with particulate material but 'ghosts', as described for *Bacillus megaterium* by Weibull (1953*b*), have not yet been identified.

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Fluorescent Metabolites of 3:4-Benzpyrene. By J. G. CHALMERS. (*Cancer Research Department, Royal Beatson Memorial Hospital, Glasgow*)

It has been demonstrated that benzpyrene added *in vitro* is associated with the lipoprotein fraction of animal serum, while after intravenous injection it is associated in metabolized form with the albumin fraction (Chalmers, 1955). An examination of the fluorescent metabolite in the serum presents some difficulty owing to the small amount of the dose present at any one time, but bile provides a better source of fluorescent material. Metabolized benzpyrene is eliminated chiefly via the bile and faeces (Peacock, 1936; Chalmers & Peacock, 1936). Tracer experiments, however, have shown that

17% of the metabolite passes into the urine (Heidelberger & Weiss, 1951), either directly or after reabsorption from the gut.

In the present experiments benzpyrene was administered by intravenous injection, intraperitoneal injection, or by feeding in the diet. Fluorescent bile was fractionated by paper chromatography using phenol as developer and showed three zones giving fluorescent spectra maxima at (4200, 4500, 4800), (4080, 4300, 4500), and (4150, 4400, 4700)Å respectively. The zones were eluted and rechromatographed using the same solvent system without any essential change in their behaviour apart from some fading of the fastest moving zone. Mouse, rabbit, guinea pig and fowl bile all gave similar fluorescence patterns. Fluorescent material separated from the faeces of benzpyrene-treated animals, and identified as 8- and 10-monohydroxybenzpyrene by Berenblum, Crowfoot, Holiday & Schoental (1943) and by Berenblum & Schoental (1946), and unchanged benzpyrene, were chromatographed in the same solvent system but behaved differently, all moving with the solvent front.

The fluorescent material in rat and rabbit urine and serum was fractionated and gave three similar fluorescent zones. In the case of rabbit urine the eluates of all the chromatographed zones gave a positive test for glucuronic acid. Also, the absorption spectrum of the zone of highest R_F value corresponded with that of 10-hydroxybenzpyrene.

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Investigations of the Chromatographic Separation of Mixtures of 4-Dimethylamino-3:5-Dinitrophenyl (DDP) Derivatives of Amino Acids on Ion-Exchange Columns. By A. Drèze* and W. S. Reith. (*Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.* 12)

Mixtures of amino acids and peptides contained in growing cells of plant roots were separated as their DDP-derivatives on cation exchange columns (Edmunds & Reith, 1954). We now find that the resolving power of the method can be considerably increased, and also that the fundamental mechanism of the ion-exchange procedure differs from that observed generally.

In a number of experiments with cation and anion exchange resins the best results were obtained with Zeo-Karb 225. The degree of cross-linking was very important. While an 8% cross-linked resin did not retain any of the derivatives used, a 4% cross-linked resin retained them all and a good chromatographic separation on this resin can be carried out; but sharper zones were obtained with a 2% cross-linked resin. A resin that was only 1% cross-linked had lost completely the power to retain any of the derivatives which were investigated.

While generally in the ion-exchange chromatography of substances described in the literature the speed of elution increases with the molar strength of the buffer, in the case of all the DDP-derivatives examined, the speed of elution *decreased* when the molar strength of the buffer increased. Monovalent cations (Na^+, Li^+) had a selective effect on the R-values of the various derivatives, while changes in the molarity of Mg^{2+} affected the R-values of all derivatives to the same extent. But also in the case of Mg^{2+} the R-values increased with the decrease in the molarity of the buffer.

There was a fair amount of adsorption on the resin; this was overcome by the addition of organic solvents to the buffer. They also influenced selectively the R-values of the various derivatives; ethanol showed the best results so far.

There was also a marked temperature effect, the R-values increasing with the increase in temperature.

The optimal conditions found so far are: 2% cross-linked resin (grain 50–100 μ.) in Na^+-form; Na acetate buffer, pH 8, molarity 1·5, containing 20% ethanol. The exchange potentials were: aspartic acid > glutamic acid > glycine > serine > alanine > threonine > valine > leucine > tyrosine > phenylalanine > basic amino acids.

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A Method of Separation of Mixtures of DDP-Ureido Derivatives of Amino Acids and of DDP-Amino Acid Hydantoins by Paper Chromatography. By A. DRÈZE* and W. S. REITH. (*Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W. 12*)

Several solvent systems can be used for the separation of mixtures of DDP-ureido amino acids by paper chromatography. However, it is necessary to use phthalate-impregnated paper so as to eliminate a marked adsorption of the derivatives, and a tailing 'downwards' of the spots. The paper strips, Whatman no. 20, were dipped in a 0·05M phthalate buffer, pH 6·0, and were dried in a current of air at room temperature.

Each solvent contained one component in which the derivatives are insoluble, and one in which they are fairly soluble.

The solvent systems for the chromatography of DDP-ureido amino acids which we found to be most useful were:

No. 1. Xylene, acetic acid, phthalate buffer (0·05M, pH 6·0); 3:2:1 by volume.

No. 2. *sec.*-Butanol, phthalate buffer; 7:1 (v/v) (used with Whatman no. 1 paper).

No. 3. Toluene, chloroethanol, pyridine, phthalate buffer; 10:6:3:6 by volume.

For the chromatography of DDP-amino acid hydantoins the most useful solvent systems were found to be nos. 1 and 2, and:

No. 4. Ligroin (b.p. > 120°), pyridine; 7:3 (v/v) (on phthalate-impregnated Whatman no. 20 paper).

For all solvents, a complete equilibration of the jars was found to be most important; with solvent no. 3 it was extremely difficult to obtain reproducible R_F values, and it was necessary to run markers simultaneously under identical conditions. The other solvents gave easily reproducible R_F values.

The R_F values were found to be independent of the amount of material used, at least between 0·01 and 1·5 μmol.

As the spots are deeply coloured, a small amount of material could easily be detected.

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DEMONSTRATIONS

Apparatus for Aeration and Sampling During Growth and Metabolic Studies with Bacteria. By E. A. DAWES and W. H. HOLMS. (*Department of Biochemistry, University of Glasgow*)

The need frequently arises for the sampling of bacterial cultures during aerated growth, or of non-proliferating bacterial suspensions during metabolic studies under aerated conditions. Two different types of apparatus have been designed to meet such requirements (Dawes & Holms, 1956).

Type 1, for bacteria which do not sediment during the period of experimentation. A 1 l. round-bottom flask with B40 neck carries a special glass head with inlet and outlet arms. The inlet tube, which extends almost to the bottom of the flask, has a sintered glass disk distributor of 20 mm. diameter and porosity no. 1. The head is held in position by spring clips. The flask is fitted with a wide-bore drain stopcock at the bottom with a stem $\frac{1}{2}$ in. long which permits samples to be withdrawn as required and without interfering with the aeration arrangements. Additionally, the flask has a B19 socket set at an angle to the neck; this is used as an inoculation port or for the addition of substrates.

Type 2 was designed for use with *Sarcina lutea* which sediments and collects in the stopcock outlet when aeration is carried out in the apparatus described above. It consists of a 1 l. capacity pear-shaped dropping funnel with B24 neck. At the base is fitted a 3-way double oblique bore stopcock with one vertical outlet $\frac{1}{2}$ in. long and the other U-shaped. The latter tube is used as the inlet for the air stream which, entering from the base of the funnel, ensures thorough agitation. By turning the stopcock the air supply is momentarily cut off, a sample is withdrawn and aeration then resumed.

Alternatively, if the air stream is replaced by nitrogen, the apparatus may be used for anaerobic studies where sedimentation is to be avoided.

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Tissue Culture Methods in Biochemistry. By J. PAUL. (*H.E.R.T. Tissue Culture Laboratory, Department of Biochemistry, University of Glasgow*)

Simple techniques will be demonstrated for the massive culture of primary explants of animal tissues and for the serial propagation of pure cell strains.

Correlated Biochemical and Histochemical Studies on Tissue Culture Explants. By J. PAUL, A. J. HALE and ENID S. PEARSON. (*H.E.R.T. Tissue Culture Laboratory and Institute of Physiology, University of Glasgow*)

Cultures of 15-day chick embryonic heart and liver were studied at daily intervals for 1 week. Samples consisting of several explants were examined simultaneously by biochemical and histochemical methods for lipid, carbohydrate and DNA. The results of the two techniques are compared and shown to complement each other.

Components of the Acid-Soluble Fraction of Mammalian Tissues. By H. M. KEIR and J. N. DAVIDSON. (*Biochemistry Department, The University, Glasgow*)

When perchloric acid extracts of rabbit tissues are adsorbed on Dowex 1 and eluted for the separation of nucleotides by the method of gradient elution described by Hurlbert, Schmitz, Brumm & Potter (1954) it has been observed that the fraction associated with cytidine monophosphate (CMP) is invariably strongly ninhydrin-positive. When this fraction is submitted to ionophoresis on paper by the method of Davidson & Smellie (1952) the ninhydrin-positive material moves well ahead of the position normally occupied by CMP. The ninhydrin-positive material on hydrolysis yields the amino acids associated with glutathione (glutamic acid, cystine and glycine) together with an ultraviolet-absorbing base.

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[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **351st Meeting** *of the Biochemical Society was held at the Rothamsted Experimental Station, Harpenden, Hertfordshire, on Friday, 25 May* 1956, *at* 10.45 *a.m., when the following papers were read:*

COMMUNICATIONS

Pyridoxal Phosphate as Coenzyme of Diamine Oxidase (Histaminase). By A. N. Davison. (*Roche Products Ltd., Welwyn Garden City*)

It has been found that the rate of inhibition of an enzyme by *iso*nicotinyl hydrazide under fixed conditions is a useful constant for identifying pyridoxal phosphate as a prosthetic group for a number of enzymes, particularly where the coenzyme is strongly bound to the apoenzyme (Davison, 1956).

Evidence presented by Sinclair (1952) suggested that pyridoxal phosphate was a coenzyme of histaminase. It therefore seemed interesting to investigate this possibility by the above method. Using cadaverine and histamine as substrates it has been found that the rates of inhibition by *iso*nicotinyl hydrazide of pig's kidney diamine oxidase are similar to that of cysteine sulphinic acid decarboxylase, an established pyridoxal phosphate enzyme. Attempts to separate a coenzyme from diamine oxidase and to reactivate the dialysed enzyme with pyridoxal phosphate failed. However, it proved possible to reactivate diamine oxidase partially with pyridoxal phosphate after the enzyme had been inhibited by *iso*nicotinyl hydrazide and dialysed.

Additional evidence was also obtained indicating that diamine oxidase and histaminase in this preparation are the same enzyme (see also Zeller, 1951). It thus seems probable that pyridoxal phosphate is coenzyme to diamine oxidase, an enzyme oxidizing both short-chain diamines and substrates such as histamine.

I am grateful to the directors of Roche Products Limited for permission to publish this work.

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The Production of Coproporphyrin Precursors by a *Rhodopseudomonas* sp. By R. Cooper (introduced by D. D. Woods). (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Free porphyrins, mainly coproporphyrin III, are excreted by suspensions of *Rhodopseudomonas spheroides* incubated in the light with glycine and α-oxoglutarate or succinate; addition of iron salts prevents the production of porphyrin but increases the formation of bacteriochlorophyll (Lascelles, 1956).

A related photosynthetic bacterium (*Rhodopseudomonas*, species not identified) has been isolated from a local pond. Suspensions of this organism incubated anaerobically in the light with glycine and succinate formed very little free porphyrin, but a colourless precursor was produced. In the presence of air the amount of precursor was reduced and considerable amounts of porphyrin appeared. Bacteriochlorophyll was formed both aerobically and anaerobically. The supernatant fluids derived from anaerobic incubations turned yellow and then red on standing in air; there was a simultaneous 30-fold increase in porphyrin content (mainly coproporphyrin III) as measured by the optical density at 403 mμ. in 1·4N-HCl. At the same time an absorption band at 500 mμ. appeared, and the optical density at this wavelength rapidly increased and then decreased as the optical density at 403 mμ. was rising. This sequence of changes was accelerated by acid, alkali, hydrogen peroxide, iodine, ferric chloride or light (especially ultraviolet), and retarded by ascorbic acid or cysteine. The supernatants were Ehrlich-negative.

A preparation of the substance absorbing at 500 mμ. was made by rapid ether extraction from 0·2N-HCl solution and subsequent precipitation from an aqueous solution at pH 3·5–4·0. A solution in 1·4N-HCl had a strong, broad absorption peak with a maximum at 500 mμ. in addition to the absorption curve of residual porphyrin. On standing the solution in air, the optical density at 500 mμ. decreased whilst that at 403 mμ. increased.

Cell-free extracts of both this organism and *Rh. spheroides* produced similar coproporphyrin precursors when incubated anaerobically with δ-aminolaevulic acid.

The colourless precursor and that absorbing at 500 mμ. may be intermediates in the conversion of δ-aminolaevulic acid to coproporphyrin.

Bogorad (1955) has recently reported that spinach leaf preparations convert porphobilinogen to a colourless product which is further converted to uroporphyrin I through an intermediate with a strong absorption band at 500 mμ.

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Cobalamin and the Synthesis of Methionine by Cell-free Extracts of *Escherichia coli*. By C. W. Helleiner* and D. D. Woods (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Gibson & Woods (1952) have shown that suspensions of a mutant strain of *Escherichia coli* deficient in cobalamin synthesize methionine from homocysteine only if cobalamin is added. Cross & Woods (1954) demonstrated the synthesis of methionine from homocysteine and serine by cell-free extracts of another mutant of *Esch. coli*, which required serine or glycine for growth. Extracts of this type were further investigated by Szulmajster & Woods (1955), but no increase in methionine synthesis occurred on the addition of cobalamin.

Cell-free extracts of a mutant of *Esch. coli* requiring either methionine or cobalamin for growth, no. 121/176 of Davis & Mingioli (1950), have now been prepared. The organisms were harvested from a medium containing a suboptimal amount of methionine, and suspensions (about 100 mg. dry wt./ml.) subjected to ultra-sonic treatment. The supernatant fluid after centrifuging was dialysed. Such extracts were more active than those obtained from acetone-dried cells.

When this preparation was incubated for 3 hr. with 10^{-2}M-DL-homocysteine, 10^{-2}M-DL-serine, 5×10^{-3}M-$MgCl_2$, 2×10^{-3}M adenosinetriphosphate, 10^{-1}M phosphate buffer (pH 7·3) and a heated extract of *Esch. coli*, from 4 to 5 μmmoles of L-methionine/mg. protein (of the enzymic preparation) was synthesized. This was increased to 15–18 μmmoles/mg. protein by adding cyanocobalamin (10 mμg./ml.). In the presence of cyanocobalamin, the omission in turn of homocysteine, serine, heated extract of *Esch. coli* and adenosinetriphosphate plus Mg^{2+} reduced methionine synthesis to about 2, 3, 4 and 8 μmmoles/mg. protein respectively.

* Post-doctoral Fellow, National Research Council of Canada.

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The Oxidation of Glucose by *Acetobacter suboxydans* By J. A. Fewster* (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Aerobic growth of *Acetobacter suboxydans* (A.T.C.C. 621) on media containing glucose and chalk (in suspension) results in the accumulation of the calcium salts of 2-oxo- and 5-oxogluconic acid in the proportion of 1:10 (see also Kulka & Walker, 1954). Washed organisms grown for 72 hr. in this manner oxidize glucose at pH 6·0 and 30° to a stage well beyond that of the oxo acids; manometric measurement shows an O_2 uptake of 3·5 moles and a CO_2 output of 4 moles/mole of substrate. Gluconic, 5-oxo- and 2-oxogluconic acid are formed, and the first two acids are oxidized in a manner compatible with their being true intermediates. The corresponding phosphorylated derivatives have not been detected and compounds representing further stages of oxidation have not been identified. 3-*O*-Methyl-D-glucose, D-glucosone, D-galactose, D-xylose and L-arabinose consume 0·5 and D-mannose 1 mole of O_2/mole; D-glucurone and glucose 6-phosphate are not attacked.

The oxidation of glucose is little inhibited by 10^{-3}M arsenite or 10^{-4}M dinitrophenol, but a concentration of 10^{-3}M dinitrophenol stops O_2 uptake at a value corresponding to the formation of the oxo acids. Appreciable inhibition results with 10^{-3}M cyanide and 5×10^{-2}M fluoride.

In agreement with Butlin (1936) it has been shown that when *A. suboxydans* is grown for 72 hr.

* Guinness Research Fellow in Microbiological Biochemistry.

on a medium containing glucose in the absence of a neutralizing agent the washed organisms possess a very limited oxidizing ability; they oxidize only glucose, with the quantitative production of gluconic acid. Full activity is not restored by addition of DPN, TPN, CoA, or a hot-water extract of organisms harvested from a medium containing chalk. In contrast to the active organisms, which are pink and rich in a single cytochrome component described by Smith (1954*a*, *b*) as being characteristic of *A. suboxydans* (α-band 554 mμ., β-band 527 mμ.), the organisms grown in the absence of chalk are cream coloured and contain, as the only cytochrome-like constituent, a small amount of material exhibiting absorption bands at 559 and 530 mμ., measured after immersion in liquid air. The presence of such a cytochrome in *A. suboxydans* has not previously been reported.

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Incorporation of ^{35}S-Sulphate by Guinea Pig Colon *in vitro*. By C. A. Pasternak, P. W. Kent and R. E. Davies. (*Department of Biochemistry, University of Oxford*)

The gastro-intestinal tract is known to be an active site of ^{35}S-sulphate fixation. Experiments on animals with intestinal fistulae given ^{35}S-sulphate intravenously have shown that the secretion of the duodenum contains a sulphated mucopolysaccharide having a high rate of turnover (Kent, Whitehouse, Jennings & Florey, 1956). The present work describes an *in vitro* technique for the study of ^{35}S-sulphate incorporated by guinea pig colon.

Lengths of colon were incubated in oxygenated Krebs–Ringer buffer containing $Na_2{}^{35}SO_4$. After 3 hr. the tissue was removed from the surrounding medium and extracted with 2% NaOH. Medium and extract were then dialysed against running tap water to remove free inorganic sulphate. Carrier Na_2SO_4 was added until constant activity was attained. Both medium and tissue extract were radioactive at the end of dialysis; this indicates the extrusion of a non-dialysable ^{35}S-labelled component by the tissue during incubation. Approximately half the total activity of non-dialysable material was in this soluble form. Gastric mucosae did not incorporate ^{35}S-sulphate under similar conditions.

Addition of 0·02M glucose to the incubation medium raised the Q_{O_2} slightly and increased the labelling of both extrudate and tissue extract several-fold (cf. Boström & Jorpes, 1954). Glucosamine, galactosamine, *N*-acetylglucosamine, potassium methane sulphonate and potassium ethyl sulphate (all 0·02M) were without effect on oxygen uptake or sulphate incorporation. Sodium fluoroacetate, phenol and salicylic acid (0·02M) abolished respiration almost completely; their action on sulphate uptake was similar. Lower concentrations of salicylic acid (0·0005–0·005M) were without effect (cf. Boström & Månsson, 1953; Kodicek & Loewi, 1955). Anaerobic incubation, and pretreatment of the tissue by heating or freezing, prevented any incorporation of ^{35}S-sulphate.

Extruded mucin in the absence of tissue did not fix ^{35}S-sulphate, which suggests that the incorporation processes are intracellular.

The ratio of sulphate incorporated/total non-dialysable sulphate was found to be higher for the extruded fraction than for the tissue extract, though in no case did it exceed 5%. Such an increase would not be detected by the analytical methods employed for the estimation of sulphate or hexosamine; it is therefore impossible to say whether the incorporation of ^{35}S-sulphate represents a synthesis of new material or a ^{35}S-sulphate exchange at pre-existing sites.

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Oxidation of Octanoic Acid by Cell-free Bacterial Extracts. By S. Dagley. (*Department of Biochemistry, University of Leeds*)

Studies with extracts from the anaerobic organism *Clostridium kluyveri* (Stadtman & Stadtman, 1953) have shown that certain fatty acids are oxidized to acetate by processes similar in essentials to those in mammalian systems, but the mechanisms employed by aerobic bacteria have remained obscure. Thus, although results with whole cells of *Serratia marcescens* (Silliker & Rittenberg, 1952) did not apparently accord with β-oxidation, it has been shown more recently that extracts from *Pseudomonas fluorescens* (Ivler, Wolfe & Rittenberg, 1955) catalyse the formation of acetate from decanoate

with the uptake of 1 μmole of O_2 per μmole of substrate. In experiments with cell-free extracts prepared from a vibrio (Happold & Key, 1932) we have now been able to show that octanoic acid is oxidized by molecular O_2 to two-carbon units; that diphosphopyridine nucleotide, coenzyme A and Mg^{2+} ions are cofactors in this process; and that subsequent oxidation most probably follows the tricarboxylic acid cycle. After growth with forced aeration in a mineral salt medium supplying octanoic acid as sole source of carbon, each 10 g. (wet weight) of cells were crushed in a Hughes press at $-14°$ and whilst still frozen they were ground in a mortar to a smooth paste with 10 g. of alumina and 25 ml. of phosphate buffer. Centrifugation to remove alumina and cell debris gave clear extracts which contained about 18 mg. protein/ml. and catalysed the oxidation of octanoate. The rate of oxidation decreased with time and the volume of O_2 utilized approached that for complete oxidation, namely, 11 moles O_2 per mole octanoate, when fumarate was added: thus 4 moles O_2 had been utilized in excess of the control after 40 min. and 8 moles O_2 after 150 min.

Uptake of O_2 by these extracts in the absence of added substrate was high, but was largely abolished by dialysis. Additions of Mg^{2+} and DPN were then necessary for activity and only 2·5–3 moles O_2 were consumed per mole octanoate. Further, volatile acid was produced in the reaction and identified as acetic acid by vapour-phase chromatography (James & Martin, 1952). Both O_2 uptake and acetate production fell somewhat short of the values required by the equation

$$C_8H_{16}O_2 + 3O_2 \rightarrow 4CH_3COOH,$$

but the acetic acid that accumulates during the reaction is inhibitory to dialysed extracts. No keto acid was detected. 'Dowex-1' treatment gave extracts whose activity was increased by addition of coenzyme A.

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Conditions for Zone Electrophoresis of Parathyroid Hormone. By A. H. Gordon. (*National Institute for Medical Research, London, N.W.* 7)

Since the successful separation of mixtures of various proteins by means of electrophoresis on columns of powdered cellulose (Porath, 1954; Campbell & Stone, 1956) it seemed possible that this method might be of value for purification of the parathyroid hormone. Although by Tiselius's electrophoresis it had previously been possible to demonstrate (L'Heureux, Tepperman & Wilhelmi, 1947) the inhomogeneity of the best preparations then available, successful purification by zone electrophoresis has not yet been achieved. Meanwhile, conditions for ultrafiltration of the hormone have been found (Davies & Gordon, 1955), and thus material of considerably lower molecular weight than that used by L'Heureux *et al.* is now available for electrophoresis.

Although powdered cellulose is often regarded as an inert support, noticeable amounts of protein can readily be shown to remain adsorbed on it even after thorough washing with buffer. Because it was feared that behaviour of this kind might be even more marked with parathyroid hormone preparations than it is with the serum proteins, an experiment to measure adsorption was carried out. For this trial 19 mg. of ultrafiltered material was applied to a column 40 × 2 cm. Recovery after elution with 0·1M-NaCl, 0·05N acetic acid was only 29%. However, when a further 7·4 mg. of the same material was applied, recovery rose to 78%, and since even higher recoveries seemed to be likely on further use of the same column, it was decided to attempt an electrophoresis. For this purpose 26 mg. of the same material dissolved now in 0·05M-NaCl, 0·05N acetic acid was used. Electrophoresis, which took place in the direction of the cathode, was carried out for 18 hr. with a current of 22 mA. Examination of the eluate proteins in the ultraviolet showed two partially resolved peaks with $E^{250}/E^{275} = 0·5$. In addition, a small amount of material with $E^{250}/E^{275} \simeq 0·9$ was found to have emerged both in front of and behind the main bands. Biological assay by the mouse urine phosphate method (Davies, Gordon & Mussett, 1955) showed that the urine phosphate activity was associated with the two main bands (tested together) and not with the material having $E^{250}/E^{275} \simeq 0·9$.

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The Sulphation of Phenol by Granulation Tissue. By G. LOEWI and P. W. KENT. (*Department of Pathology, Radcliffe Infirmary, Oxford and Department of Biochemistry, University of Oxford*)

Kodicek & Loewi (1955) have reported the formation *in vitro* of a sulphated mucosubstance by granulation tissue. It was therefore of interest to determine whether this tissue was also capable of synthesizing other *O*-sulphates. The conjugation of phenol as the sulphate and as the glucuronide was demonstrated by De Meio & Arnolt (1944) in liver. No such conjugates appear to be formed by spleen, heart, kidney or hepatoma (Segal, 1955). Dodgson & Spencer (1954) have described a chondrosulphatase and an aryl sulphatase in the periwinkle (*Littorina littorea*).

Granulation tissue (Kodicek & Loewi, 1955) was sliced free-hand and incubated at 37° in Krebs–Ringer buffer (pH 7·4) containing 4 μc $Na_2{}^{35}SO_4$, 0·1 % (w/v) glucose and 10^{-3}M phenol (final concentration) in pure oxygen. After shaking for 3 hr., the tissue was removed, dried and weighed. The residual medium, after extraction several times with ether to remove free phenol, was concentrated to dryness under reduced pressure. The residue was extracted with ethanol and the resulting extract was examined chromatographically on Whatman no. 1 paper using *n*-butanol–ethanol–water (4:1:5, v/v).

After treatment with HCl gas and heating at 100° the papers were sprayed with a solution of diazotized sulphanilic acid and exposed to ammonia vapour. The media extracts showed a single component as an orange-yellow spot, R_P 0·47 (identical with authentic phenol sulphate) and having, in different experiments, a radioactivity of between 200 and 300 counts/min. The same compound was obtained from liver slices but with approximately ten times greater radioactivity. When non-radioactive phenol sulphate was added to the ethanolic extracts, one coincident coloured and radioactive component (R_P 0·47) was found. Inorganic sulphate ions were immobile in this system.

No phenol sulphate was detected when phenol was omitted from the incubation medium nor when boiled tissue was employed. The presence of phenol did not diminish the Q_{O_2} of granulation tissue.

It is concluded that granulation tissue is capable of sulphating not only carbohydrate substrates but also phenol. Further work is necessary to show the nature and relationship of the enzymes involved.

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Biochemical Genetics of the Flower Pigments of *Primula sinensis*. By THELMA O. DAYTON, J. B. HARBORNE and H. S. A. SHERRATT. (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

The biochemical genetics of the flower pigments of *Primula sinensis* was earlier investigated at the John Innes Horticultural Institution (Scott-Moncrieff, 1936). Red flowers (*K*) contained malvidin 3-galactoside which was dominant to pelargonidin 3-glycoside in orange flowers. A factor *Dz* controlled the independent formation of pelargonidin in the presence of *K*. Other genes influenced flower colour by the production of a flavone co-pigment, and by altering the pH of the cell sap and the concentration of anthocyanin.

A reinvestigation of different genotypes of *P. sinensis* by modern methods has revealed a more complex situation. In general, *K* types contain malvidin, delphinidin and petunidin; these may be present as more than one glycoside. Two glycosides of kaempferol are present, only one of which appears to act as a co-pigment. An unidentified flavanone glycoside has also been isolated in crystalline form. The names sinensetin and sinensin are provisionally proposed for the aglycone and glycoside respectively. Sinensetin from its absorption spectra in neutral and alkaline solutions and its colour reactions, does not appear to be related in its *A*-ring hydroxylation pattern to any of the other phenolic pigments present, even though it apparently competes with these other pigments for precursors. This is in contrast to the situation in *Antirrhinum majus* in which plant all the pigments present are closely related in structure and in gene control (Geissman, Jorgensen & Johnson, 1954; Dayton, 1956).

The genetical results (Dayton, 1954) show some new interactions between the various genes controlling pigment production which can be interpreted in terms of competition for precursors.

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Biochemical Genetics of the Acylated Anthocyanins of Tuberous Solanums. By J. B. HARBORNE (introduced by H. S. A. SHERRATT). (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

As part of an investigation of the inheritance of colour in potatoes, Dodds & Long (1955) found acylated glycosides of pelargonidin, paeonidin and petunidin to be the main pigments present in cultivated diploid species. The first two anthocyanins were present in red tubers (*ppRR*) and the last two in blue tubers (*PpRR*). The presence of a gene for acylation, which behaved as a simple Mendelian factor, was later observed by the same authors (unpublished work). Plants with non-acylated pigments had either deep red or deep blue tubers and flowers according to their genotypes.

The pigments of the red, deep red, blue and deep blue tubers have now been studied in detail, using chromatographic and spectrophotometric methods, as described for similar investigations of phenolic pigments (Geissman, Harborne & Seikel, 1956). Acid and alkaline hydrolyses of the carefully purified eluates of the separated pigments gave information regarding glycosidation and acylation respectively. The results obtained indicate that the acylating gene has at least four biochemical effects on pigment production. Besides controlling acylation of the anthocyanins with *p*-coumaric acid, it changes the positions of the sugars (arabinose and glucose) from 3-pentose glycoside in the recessive state to 3:5-dimonoside. Also in its recessive state, the production of paeonidin is suppressed and instead cyanidin is formed in deep red tubers, delphinidin in deep blue tubers.

Measurements of the absorption spectra of the purified anthocyanins in the range 250–600 mμ. indicate not only the presence or absence of the acyl group but also that the ratio of *p*-coumaric acid to anthocyanin is 1:1. Other results suggest that the acyl component is linked to the anthocyanin through a sugar residue.

Cyanidin in a non-acylated form, probably as the 3-rhamnoglucoside, and rutin (quercetin 3-rhamnoglucoside) occur chiefly as flower pigments in plants of all four of the above genotypes. This suggests that in potatoes the biosynthetic pathway to cyanidin diverges at an early stage from the pathway to the other anthocyanins present.

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Genetics of Pathogenicity in *Fusarium oxysporum*. By E. W. BUXTON (introduced by R. H. KENTEN). (*Rothamsted Experimental Station, Harpenden, Herts*)

Irradiating conidia of *Fusarium oxysporum* f. *pisi*, the cause of pea wilt (Linford, 1928; Snyder, 1935; Buxton, 1955), with ultraviolet light alters their nutritional requirements *in vitro*, and these can be classified into specific needs for amino acids, yeast nucleic acids or vitamins. Fifty-one mutants, derived from analysis of 2119 irradiation survivors, were used as genetic markers for synthesizing heterokaryons (colonies with different nuclear types associated in the same hyphae) between differently virulent strains of the fungus.

Avirulent mutants formed heterokaryons, by germ tube anastomosis (Buxton, 1954), which had virulence restored to the same level as that of the original pathogenic wild type isolates. When heterokaryons were made between nutritional mutants derived from pathogenic races 1 and 2 (distinguished by their abilities to wilt different pea varieties), three prototrophic conidia occurred in 10^8 conidia from a heterokaryon. These three conidia were easily recognizable because they grew like wild types on non-supplemented medium. Progeny from the prototrophic colonies contained a small proportion of segregants and recombinants which were detected by their nutritional requirements, their colour on a non-nitrogenous modified Czapek-Dox agar medium, and their differential tolerance towards an actinomycete inhibitor (Buxton & Richards, 1955). When recombinants were tested for pathogenicity on pea varieties known to differentiate the three physiologic races of the fungus, some wilted a variety resistant to races 1 and 2, thus resembling the wild type race 3. This suggests a possible mode of origin of physiologic races in *Fusarium*. The underlying genetic mechanism, which resembles the para-sexual cycle described in *Aspergillus* and *Penicillium* spp. (Pontecorvo, Roper & Forbes, 1953; Pontecorvo & Sermonti, 1954), together with the effect of heterokaryosis on virulence, might explain much of the variability prevalent in the pathogenicity of this and certain other Fungi Imperfecti.

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The Effect of Nitrogen Mustards on the Incorporation of Labelled Amino Acids into Protein by *Staphylococcus aureus*. By A. R. CRATHORN and G. D. HUNTER. (*The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.* 3)

The modes of action of radiomimetic agents employed in cancer therapy are not well understood. In the course of a search for a system where direct biochemical effects could be demonstrated, the action of nitrogen mustards on the utilization of amino acids by *Staphylococcus aureus* Duncan has been investigated.

Gale & Folkes (1953) have shown that in addition to the normal assimilation of amino acids that takes place under conditions of net protein synthesis (condition 1), a further type of amino acid incorporation into protein can occur in this organism. In the absence of a complete source of essential nitrogen compounds, individual amino acids are incorporated into protein by *Staph. aureus* by what appears to be an 'exchange reaction' with amino acid residues in the protein molecules already present (condition 2). Our results show a marked correlation between the inhibition of amino acid incorporation under condition 2 and the activity of the nitrogen mustards in causing tumour inhibition. Under the same conditions and time of exposure to the chemical agent, the effect of even the most active nitrogen mustards on incorporation of amino acids under condition 1 is small. Similarly, the inhibitory effects obtained are not explicable in terms of a loss of viability.

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Isolation of D-*glycero*-D-*galacto*-Heptose from the Specific Polysaccharide of *Chromobacterium violaceum* (BN). By A. P. MACLENNAN and D. A. L. DAVIES. (*Microbiological Research Establishment, Porton, Wilts*)

Only in recent years have aldoheptose sugars been found in nature (Jesiatis & Goebel, 1952; Slein & Schnell, 1953; Fromme, Lüderitz & Westphal, 1954; Weidel, Koch & Bobosch, 1954; Davies, 1955; Davies, Morgan & Record, 1955; Crumpton & Davies, 1956; Davies, 1956). In only one of these investigations (Slein & Schnell, 1953) was the sugar identified (L-*glycero*-D-*manno*-heptose).

The specific polysaccharide of *Chromobacterium violaceum* (BN) contains an aldoheptose sugar (Davies, 1955). A preparation of the somatic antigen of this organism was obtained from acetone-dried bacteria by extraction with trichloroacetic acid (Boivin, Mesrobeanu & Mesrobeanu, 1933). A degraded polysaccharide hapten obtained from the antigenic complex was shown by paper chromatography to have three components, an amino sugar, a methyl pentose and an aldoheptose.

A salt-free hydrolysate of the polysaccharide was placed on a resin column (Zeo-Karb 225) and the neutral sugars were eluted with water. The amino sugar was subsequently eluted with 0·3N-HCl, crystallized and identified as D-glucosamine. The solution of neutral sugars was placed on a column of cellulose powder; on eluting with 95% (v/v) acetone the methylpentose emerged first. This was crystallized and identified as L-rhamnose. A later fraction which contained the aldoheptose was evaporated to a syrup and a sample, after drying under acetone, showed a rotation, $[\alpha]_D^{20} = +61°$. This approached the value of D-*glycero*-D-*galacto*-heptose ($+68·6°$) but differed from the values recorded for other heptose sugars. In a variety of solvent systems the sugar corresponded in position on paper chromatograms with D-*glycero*-D-*galacto*-heptose and differed in position in at least one solvent system from seven other synthetic heptoses which were available for comparison. Crystallization proved difficult, but a small yield of heptose crystals was finally obtained from 90% (v/v) ethanol. The infrared absorption spectrum of the isolated sugar differed markedly from those of seven different aldoheptoses but was identical with that given by an authentic sample of D-*glycero*-D-*galacto*-heptose.

We are greatly indebted to Professor N. K. Richtmyer for generous samples of aldoheptose sugars, and to Mr L. C. Thomas for carrying out the infrared spectroscopy.

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The Biosynthesis of Ergothioneine by *Claviceps purpurea*. 2. The Incorporation of [^{35}S]Methionine and the Non-Utilization of [2-^{14}C]Histamine. By JENNIFER WILDY and H. HEATH. (*University College Hospital Medical School, London, W.C.* 1)

The incorporation of [2-^{14}C]acetate into ergothioneine by *Claviceps purpurea* has been previously reported by Heath & Wildy (1956). This showed clearly that there was a direct relationship between ergothioneine and histidine biosynthesis. We now wish to report further work on the use of other potential precursors.

[^{35}S]Ergothioneine was isolated from a 10-day shake culture of *C. purpurea* grown as previously described. The sulphur source consisted of $MgSO_4$ (0·03%) and [^{35}S]methionine (0·004%). The biosynthetic pathway was not further elucidated, however, by this incorporation of isotope since this fungus is capable of utilizing sulphate for its entire sulphur requirements and it was shown that part, at least, of the labelled methionine had been oxidized to sulphate in the medium. Thus the isotope in the ergothioneine could have been derived from sulphate or any of the other sulphur compounds which are themselves derived from sulphate, and not necessarily from the labelled methionine. The addition of inactive pools of sulphur compounds adversely effected the growth of the fungus and the identity of the immediate sulphur precursor of ergothioneine has not so far been established.

From our previous work on the incorporation of [2-^{14}C]acetate it was apparent that the synthesis of ergothioneine and histidine were closely related. As [2-^{14}C]histamine was readily available and histamine is itself a constituent of ergot, the effect of this compound on the system was studied. Both the ergothioneine and histidine isolated from a culture of *C. purpurea* grown on [2-^{14}C]histamine were unlabelled. Although histamine is not a precursor of ergothioneine this work has thrown light on the metabolism of the imidazole ring system by this fungus because all the residual activity was associated exclusively with imidazole compounds. There was no degradation of the imidazole to yield a one carbon fragment derived from the C-2 of the ring. Neither did free histamine remain in the culture broth; the majority of the activity was found in at least three diazo-reacting compounds. Paper chromatography of the broth before and after hydrolysis showed that one compound was imidazoleacetic acid but the other two compounds could not be identified. The recent publication of Karjala, Turnquest & Schayer (1956) shows that the principal urinary metabolites of histamine in the mouse, besides imidazoleacetic acid, are 1-ribosylimidazole-4-acetic acid and 1-methylimidazole-4-acetic acid.

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An Indole–Alkylamine Regulating Heart-beat in Crustacea. By D. B. CARLISLE. (*The Plymouth Laboratory of the Marine Biological Association of the United Kingdom*)

The pericardial organs of Crustacea are neurohaemal organs consisting primarily of nerve endings, with some connective tissue, and lie free in the blood. Histological evidence suggests that they secrete a neurohumour into the blood. Extracts of these organs contain a principle which regulates heart beat. There is evidence that this substance is in fact released into the bloodstream and removed from the blood by the heart. Addition of an equal amount of N-NaOH to an extract under aerobic conditions leads to a transient green fluorescence, similar to that given by catechol amines. The activity is destroyed by amineoxidase and more slowly by *ortho*-diphenol oxidase, prepared from potatoes. Of

a large range of amines tested on crustacean hearts only 5-hydroxytryptamine had a similar effect under all conditions to the extract of pericardial organs. Paper chromatography yielded two spots, one with a lower and one with a higher R_F value than 5-hydroxytryptamine, but gave no evidence for the presence of this substance. On elution both of these spots proved active. Both gave the same colour reactions as 5-hydroxytryptamine and are certainly indole-alkylamines. One is destroyed by *ortho*-diphenol oxidase and may be presumed to be an *ortho*-dihydroxyindole-alkylamine. The other is destroyed by this enzyme, but far more slowly. If a fresh extract is left standing the second substance disappears to be replaced by a roughly equivalent amount of the first substance. This may be the precursor substance, presumably with one of the hydroxyl groups blocked in some way. It seems most plausible from the biological evidence taken in conjunction with the pharmacological and biochemical evidence that the active substance is an *ortho*-dihydroxytryptamine.

DEMONSTRATIONS

The Rothamsted Farm. By H. V. Garner (introduced by R. H. Kenten). (*Field Experiments Section, Rothamsted Experimental Station, Harpenden, Herts*)

The Rothamsted Experimental Station was founded by Sir John Bennett Lawes, a Hertfordshire landowner, in 1843. He had already set himself up as a fertilizer manufacturer and taken out a patent for superphosphate—his own invention. He had the vision to foresee the importance of inorganic salts as sources of plant nutrients, and at the same time he had the agricultural knowledge which convinced him that the most practical way of studying crop nutrition and impressing his findings on his fellow farmers was to set up a system of field experiments designed to last for a period of years.

Thus began the classical experiments on wheat, barley, mangolds and grass which still survive. Rothamsted is now a major centre of field experimentation in this country, and it possesses not only the unrivalled series of old plots with their vivid contrasts due to long periods of differential manuring, but also numerous experiments based on statistical layouts.

The Rothamsted farm in early summer shows the effects of nitrogenous fertilizers on cereals and grass, as they occur on the old fields. The effects of phosphorus and potassium are less visible at this stage, though the classical barley plots generally show earlier growth where phosphate is supplied.

The layout can be seen of more complicated experiments in which the comparison lies not between fertilizers but between systems of farming. Briefly, the problem is to determine whether one kind of rotation maintains soil structure and soil nutrients more than another. Any lack of soil nutrients can easily be repaired but the loss of structure can be a much more serious matter.

Many of the experiments deal with the control of plant diseases caused by virus and fungi and are closely linked with the laboratory activities of the biological staff.

Apart from the plots, which number about 2000, there is a large range of buildings equipped with the machines and facilities necessary for experimental work, and a productive commercial farm, whose fields can be drawn on for further experiments as required.

The Large-Scale Production of Leaf Protein. By M. Byers, D. Fairclough and N. W. Pirie. (*Rothamsted Experimental Station, Harpenden, Herts*)

Our aim is to separate protein from the fibrous and strongly flavoured components of the leaf and so make, from material suitable only as food for ruminants, a food for non-ruminants including man. The by-products will also be used, but the demonstration is limited to protein. Most types of fresh, lush leaves can be used and extraction is as a rule better the greater the water and protein content of the leaf.

Pulping takes place in a drum containing beaters fixed to an axial shaft driven at 400–1500 r.p.m. according to the texture of the crop. The rate of movement through the drum is controllable so that the amount of work done can be varied with consequent variation in the extent of extraction and the quality of the protein extracted.

Pulping consumes 3–10 kwh./ton of wet leaf and efficiency depends on optimal feeding. The feeder is

therefore controlled by relays in the circuit of the pulper motor so that the feed varies and keeps power consumption fairly constant. The pulp falls on to a perforated conveyor belt and travels into an automatic hydraulic press. In this, the conveyor moves only when no pressure is on it and pressure can be maintained for as long as necessary. The first factor makes this press superior to screw expellers and the second to presses in which rollers press the material. About a third of the protein comes out in the juice and a further quantity comes out if the extraction is repeated.

Leaf proteins coagulate between 50° and 80°. Fractions with different properties can therefore be made, but for convenience we heat continuously with steam to 75–80° in an automatic unit which adjusts inflow to keep outflow temperature constant. Alternatively, protein can be coagulated by ageing, acidification or shaking with water-immiscible solvents. After heating, protein filters off easily in vertical cloth sleeves. The mass of protein is pressed to 40% DM, shredded, suspended in water and pressed again.

In this form it has to be kept under refrigeration but can be kept at room temperature if dried or extracted with acetone or methanol. After extraction it is pale green or fawn, tasteless, and contains 10–13% N, i.e. it is 60–80% protein. The remainder is starch, fat, fibre and minerals in varying amounts depending on the original crop and the care exercised in the preparation.

Electron Microscopy. By H. L. Nixon (introduced by N. W. Pirie). (*Rothamsted Experimental Station, Harpenden, Herts*)

The microscope is an RCA type B instrument with many home-made modifications; the resolving power under everyday conditions is about 30–40 Å, with a best performance of perhaps 20 Å. The Metropolitan-Vickers evaporating plant has been modified to make it suitable for making evaporated carbon replicas and support films in addition to the usual shadow-casting with platinum or palladium. There is a Cook and Perkins ultramicrotome.

1. *The use of electron microscopy for counting virus particles.* The spray droplet method of Backus & Williams (1949) has been used in few laboratories because of practical difficulties. These have been overcome by modifications to the original method, and particle counting by electron microscopy is now used, together with serological and infectivity tests, in studies of virus multiplication under different conditions.

Counting is particularly useful at low concentrations, and solutions containing down to 0·1 mg./l. or about 10 particles of tobacco mosaic virus (TMV)/ml. can be assayed without preliminary concentration. This is below the limit for serological tests. With higher concentrations there is excellent agreement of the counts with the end points of precipitin tests made with the same samples from a dilution series of purified TMV.

2. *Thin sectioning of plant tissue.* The standard techniques for ultra-thin sectioning are difficult to apply to plant material because penetration of solutions into the tissues is very slow, even from cut edges. This makes the use of very narrow strips essential, and, even so, preservation is worse than that obtained with animal tissues. In making a study of the way in which the submicroscopic structures in the cell are affected by virus infection, the aim is to find the site or sites of virus multiplication within the cell. Characteristic changes in the internal structure of some chloroplasts in tobacco leaf parenchyma cells have been observed in tissue from plants carrying a systemic infection of TMV. Progress is slow, because TMV rods do not stain with osmium from the fixative and are therefore difficult to see in sections examined with the methacrylate embedding still in place. If the embedding material is removed by solution and the preparation shadowed, the rods can be seen easily, but the process of solution destroys fine structure and makes interpretation difficult.

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Pot Culture and Solution-Culture Experiments in Glasshouses. By D. J. WATSON (introduced by N. W. PIRIE). (*Rothamsted Experimental Station, Harpenden, Herts*)

The pot culture experiments deal with the following subjects:

(1) An attempt to diagnose the nutrient status of soil by measuring nutrient uptake of excised roots transferred from the soil to standard laboratory conditions; the material used is barley grown in Rothamsted soil with varying supply of N, P and K fertilizers.

(2) Onset of dormancy in the seeds of the winter wild oat (*Avena ludoviciana*) during the ripening of the panicles.

(3) Effect of applying nitrogenous fertilizer to barley at sowing on the response to a late N application at ear emergence.

(4) An analysis of the effect of N, P and K on leaf growth of sugar beet, in terms of the rate of production, longevity and size of leaves.

(5) Effect of variations of soil moisture content between field capacity and wilting on the growth of sugar beet.

The solution-culture experiments are concerned with:

(1) The effect of excising root tips or varying fractions of the root system on subsequent growth of roots and on nutrient uptake.

(2) Influence of boron on transport of carbohydrate from leaves.

(3) Use of various iron complexes as sources of iron in culture solutions of varying pH.

(4) Interaction of molybdenum with iron supplied in different forms (chloride, citrate, Fe-EDTA).

Aphid Dispersal. By C. G. JOHNSON (introduced by N. W. PIRIE). (*Rothamsted Experimental Station, Harpenden, Herts*)

Trapping techniques. During recent years we have employed various methods in the trapping of airborne insects at different altitudes, e.g. nets on a barrage-balloon cable up to 4000 ft. and electrically operated suction traps. One of the latter is on display; it consists of a fan sampling air at a constant rate. The insects in the air are deposited in a tube; disks drop automatically into this tube, so dividing up the catch at pre-set time intervals.

Flight of aphids from the crop. Photographs show the fluctuations in numbers of aphids flying from hour to hour over a crop heavily infested with aphids. There is typically a bidiurnal periodicity of flight.

Dispersal in the upper air. Aphids become generally dispersed by turbulent diffusion to great heights in the air. The density diminishes with altitude. It has been necessary to construct extremely large and powerful traps in order to sample adequately the low concentrations of aphids found at the higher altitudes. Several traps sampling at different altitudes simultaneously (up to 1000 ft.) enable the aphid density gradient to be estimated. Diagrams show that in general the air is populated and depopulated at least once in 24 hr.

Flight experiments are in progress to assess the temperatures at which aphids cease to fly; an aphid fastened by gum on to a pin is allowed to fly in an air stream whose temperature is lowered gradually. The rate of cooling strongly affects the temperature at which flight ceases.

Aphids are weak fliers, therefore the direction of the wind determines their direction of travel. This is reflected in the intensity of infestation on the windward edges of fields.

Insect Rearing and Methods of Testing Insecticides. By the Staff of the Department of Insectides and Fungicides (communicated by K. A. LORD). (*Rothamsted Experimental Station, Harpenden, Herts*)

Satisfactory control of insect pests by chemicals is only possible if suitably toxic chemicals are available and can be administered economically in sufficient quantity without causing harm to man, crops or farm animals.

In practice insects may acquire a toxic dose of insecticide as a vapour, by ingestion of contaminated food or by contact with the substance, when it is absorbed through the cuticle.

A variety of laboratory techniques for the bioassay of insecticides is demonstrated. The equipment makes it possible to administer controlled doses of insecticides by various routes and under different conditions.

Potter tower. A device for applying a uniform film of insecticide over a 9 cm. Petri dish, mainly used for the comparison of the toxicity of contact insecticides either as direct sprays or residual films.

Dipping apparatus. Equipment devised to test changes in toxicity of large crystals broken up by spray nozzles. It can be used in other work on the action of contact insecticides.

Topical application. Measured drops of insecticide are placed on the insect cuticle and the toxicity of insecticides by direct application may be measured. This method has been used to investigate the synergistic activity of piperonyl butoxide and sulphoxide on the pyrethrins. This apparatus may also be used for the subcuticular injection of poisons.

Stomach poison tests. Equipment used for assessing the toxicity of substances ingested by insects, avoiding as far as possible contact action.

An adequate supply of healthy test insects is essential to bioassay and the rearing of some of the insects frequently used in the department is illustrated. These include:

Diataraxia oleracea (tomato moth); *Gryllus domesticus* (cricket); *Blattella germanica* (German cockroach); *Musca domestica* (house flies); *Blatta orientalis* (oriental cockroach); *Periplaneta americana* (American cockroach); *Acyrthosiphon pisum* (pea aphid); *Megoura viciae* (vetch aphid); *Dysdercus fasciatus* (cotton stainer); *Pieris brassicae* (cabbage whites); *Phaedon cochlcariae* (mustard beetle); *Drosophila melanogaster* (fruit fly); *Ephestia kühniella* (grain moth); *Tenebrio molitor* (mealworm); *Oryzaephilus surinamensis* (saw-toothed grain beetle); *Tribolium castaneum* (red rust flour beetle); *Calandra oryzae* (rice weevil); *Achroia grisella* (wax moth); *Trogoderma granarium* (Khapra beetle); *Oryzaephilus mercator* (merchant grain beetle).

Insect pests may be controlled by destruction of the eggs by the application of chemicals. Frequently the embryos appear to develop normally but fail to hatch.

Studies on the toxicity of insecticides to eggs are illustrated in relation to the development of esterases.

A Model to Demonstrate Separation in Double Helices. By C. O. Person (introduced by H. S. A. Sherratt). (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

The model consists of three parallel rods, each of which is free to rotate. A double helix, formed of cotton tape wound about the centre rod with free ends attached one to each of the side rods, is unwound and two single helices are simultaneously formed when the three rods are rotated.

Deoxyribonucleic acid (DNA) is usually considered to have a molecular structure consisting of two complementary, helical polynucleotide chains related to a common axis (Watson & Crick, 1953). A difficulty encountered in the interpretation of DNA replication, supposed to occur in living cells, is in accounting for the separation of the two chains so that *each* chain may then act as a template for the formation of a new double helix. A process of rotation similar to that achieved by the model, if proposed for the reproduction of the DNA molecule, would require that the helix be free to rotate, and that complementary chains form on to, and impose, a helical structure on the separating DNA chains. This mechanism would be simpler than others that have been advanced previously (Delbrück, 1954; Gamow, 1955).

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Taurine-S35
Glycerol-1-C14
DL-Serine-3-C14
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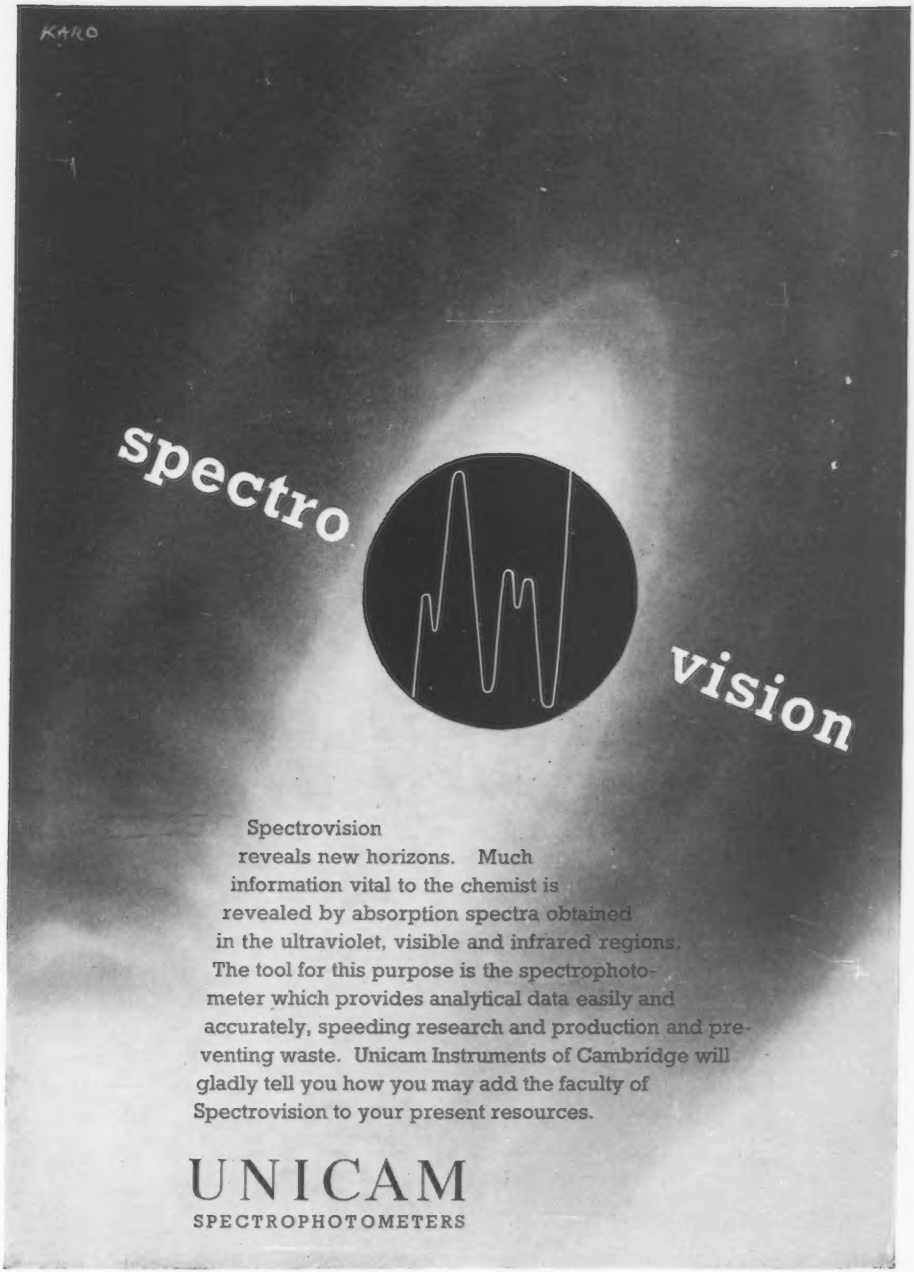

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held at University College, Gower Street, London, W.C.* 1, *on* 22 *March* 1956, *at* 10.45 *a.m., when the following papers were read:*

COMMUNICATIONS

The Composition of Some Protein Fractions Extracted from Calf Skin. By J. H. Bowes, R. G. Elliott and J. A. Moss. (*British Leather Manufacturers' Research Association, Egham, Surrey*)

During investigations on the extraction of acid-soluble collagen from skin, indications of the presence of a non-collagenous protein constituent closely associated with the collagen were obtained (Harkness, Marko, Muir & Neuberger, 1954; Bowes, Elliott & Moss, 1955). Further work on the exhaustive extraction of calf skin with dilute acid and alkaline solutions has now furnished evidence for the presence of two non-collagenous protein fractions in this tissue.

The middle layer of a calf skin was disintegrated and extracted successively with six changes of each of the following solutions: phosphate buffer, pH 9·1; citrate buffer, pH 3·7; dilute acetic acid, pH 2·8; and sodium hydroxide, pH 12·2. The bulk of the protein extracted by the dilute acid solutions was precipitated by sodium chloride, and hydroxyproline and tyrosine determinations indicated that it was mainly collagen. The filtrates were dialysed and concentrated. The composition of the two protein fractions so obtained differed considerably from that typical of collagen; in particular, the hydroxyproline content was negligible, the glycine, proline and arginine contents were lower, and the tyrosine, histidine, leucine, isoleucine, valine, dicarboxylic acids and amide contents were higher than in collagen. The composition of the two fractions differed slightly, but there was insufficient evidence to decide whether they were two different proteins, or different degradation products of the same protein.

The fraction isolated from the alkaline extracts by precipitation with ethanol was also found to consist mainly of non-collagenous-type protein. Its composition was similar to those of the acid-soluble non-collagenous fractions, but there were certain differences. Both the acetic acid-soluble and the alkali-soluble fractions were associated with relatively large amounts of hexosamine.

Thus, two relatively soluble non-collagenous proteins appear to be closely associated with the collagen in skin. The alkali-soluble fraction may represent the protein moiety of the mucopolysaccharide of the ground substance. The hydroxyproline content of the extracted calf skin indicated that it still contained about 7 % of a non-collagenous protein. This protein fraction must be closely associated with the collagen and may even form an integral part of the collagen fibres. It is possible that the acid-soluble non-collagenous fractions represent soluble forms of this component.

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The Biosynthesis of Ergothioneine by *Claviceps purpurea*. I. The Incorporation of [2-^{14}C]-Acetate. By H. Heath and Jennifer Wildy. (*University College Hospital Medical School, London, W.C.* 1)

Claviceps purpurea, growing parasitically on rye, forms sclerotia which contain alkaloids and ergothioneine. This fungus has been grown on numerous solid and liquid media; in no case have sclerotia been formed and neither alkaloids nor ergothioneine could be detected. After repeated subculturing, ergothioneine was formed in cultures grown on a mannitol, ammonium succinate, sulphate, phosphate and trace metal medium and was labelled by the addition of 1·0 mc [2-^{14}C]acetate. Ergothioneine was isolated by ion exchange and alumina (Melville & Horner, 1953) chromatography and crystallized to constant activity. It was degraded as follows: ergothioneine, trimethylamine and thiolurocanic acid; thiolimidazole propionic acid; imidazole propionic acid; ethyl imidazole propionate; ethyl 4:5-dibenzamidopentenoate and sodium formate; carbon dioxide and

3,4-dibenzamidobutene. All compounds except the last, which was not isolated, were converted to $BaCO_3$ prior to counting.

The highest activity (2411 c.p.m./mg. C) was present in C-2 of the ring, followed by the methyl carbons (500 c.p.m./mg. C) of the betaine; the five-carbon residue had only 113 c.p.m./mg. C, the carboxyl carbon accounting for at least 60% of this activity. The low activity of the five-carbon chain of ergothioneine shows that glutamate, derived from acetate via the citric acid cycle, is not the precursor of this chain. Thus there are similarities between the pathways of histidine and ergothioneine biosynthesis.

At present, the precursors of the five-carbon chain in histidine are not definitely known. Glutamate is not the precursor as [2-^{14}C]acetate is not incorporated into this chain by yeast (Levy & Coon, 1954). Ehrensvard, Reio, Saluste, & Stjernholm (1951) showed, however, that with yeast grown on acetate as the sole carbon source the carboxyl carbon of histidine is derived from the methyl carbon of acetate.

Support for a pentose pathway is given by Ames & Mitchell (1955), who showed that histidine mutants of *Neurospora* accumulated imidazole-glycerol, imidazole-acetol and histidinol.

Work is in progress to see whether the formation of the thiolimidazole moiety occurs by addition of sulphur to the preformed imidazole ring system or whether the sulphur is attached to the ultimate C-2 of the ring before ring closure occurs.

The authors are very grateful to Messrs Burroughs Wellcome Ltd for a gift of ergothioneine.

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Detection of Tissue Clearing Factor by its Effect on Lipoprotein Electrophoresis. By A. Comfort.
(*Department of Zoology, University College, London, W.C.* 1)

Korn (1955) has found that normal tissues, and acetone powders prepared from them, contain a substance which appears identical with the clearing factor released into the plasma by injected heparin. We have used the selective increase in the electrophoretic migration of serum lipoproteins to detect response to heparin in lipaemic rabbit serum, which does not undergo clearing (Comfort, 1955). This is a sensitive test either for clearing factor or for something closely associated with it.

Fresh brei and acetone powders from several tissues increase the migration of added plasma lipoprotein. Tissues were minced with scissors; powders were prepared by homogenizing in acetone at $-20°$ and drying at room temperature. Human plasma was used as substrate. Each batch was divided into many 5 ml. portions which were frozen at $-20°$ and thawed once only as required. Fresh brei or acetone powder was ground with 2 ml. test plasma, centrifuged after 2 hr. at room temperature, and examined electrophoretically, after dilution with more test plasma if necessary. A rough quantitative estimate of activity was given by comparison with standards.

Lipoprotein migration was selectively increased by fresh and acetone-dried mouse, rabbit and duck heart and lung, fresh rabbit kidney and lymph gland, but not by skeletal muscle or frog lung, liver, heart, testis, caecum or muscle.

Korn (1955) extracted clearing factor from acetone powders with serum or 0·025N-NH_4OH. Although activity detectable both by optical clearing and by lipoprotein acceleration is extracted by alkali, the bulk of the activity, judged by the second test, remains attached to the solid residue. This does not indicate that tissue factor is particulate, since it is equally hard to elute from alcohol precipitates of serum proteins. The identity of clearing and accelerating substances cannot be assumed, but they show no tendency to separate.

We found no evidence of interference by other tissue constituents. Comparison of parallel runs with successive dilutions of the factor indicates that it produces a simple increase in the anodic migration of one or both lipoprotein bands, not a transfer of lipoid from one serum protein to another (Brown, Boyle & Anfinsen, 1953). Action on the α-lipoprotein is inconstant—albumin mobility is sometimes increased, probably as a result of the binding of fatty acids released by the factor or an accompanying lipase (Ballou, Boyer & Luck, 1945).

This work was done under a Fellowship awarded by the Nuffield Foundation.

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The Chemical Nature of Tetrahydroxynorsterocholanic Acid. By G. A. D. HASLEWOOD (*Guy's Hospital Medical School, London, S.E.* 1) and I. D. P. WOOTTON (*Postgraduate Medical School, Ducane Road, London, W.* 12)

The above acid was isolated by Ohta (1939) from the bile of the Japanese 'Gigi' fish, and since it has been found in the bile of other teleostean fish and also in that of chickens (Yamasaki, 1951), it is probably a 'primitive' bile acid of widespread occurrence.

Ohta (1939) and Isaka (1940) after a study of its chemistry concluded that tetrahydroxynorsterocholanic acid, $C_{27}H_{46}O_6$, was a 3:6:12:24-tetrahydroxy 'coprostanic acid', and could be degraded to 3:6:12-trihydroxycholanic acid. Attempts at partial synthesis of 3α:6α:12α-trihydroxycholanic acid have given material not having the properties mentioned by Isaka (1940) and we decided to test by direct examination the formula of Ohta and Isaka. A sample of tetrahydroxynorsterocholanic acid, m.p. 215–217°, kindly given by Professor T. Kazuno of Okayama Medical School, was methylated and examined by infrared spectroscopy. In the region 900–1300 cm.$^{-1}$, the spectrum closely resembled that of methyl cholate (3α:7α:12α-trihydroxycholanate). According to observations previously made (Wootton, 1953), we conclude that tetrahydroxynorsterocholanic acid almost certainly has the cholic acid nucleus, i.e. it resembles all other 'primitive' bile acids or alcohols so far examined in being a 3α:7α:12α-trihydroxycholane derivative.

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The Speed of Oxidation of Reduced Nicotinamide Nucleotides by Cerebral Preparations. By H. MCILWAIN and M. A. TRESIZE. (*Department of Biochemistry, Institute of Psychiatry, British Postgraduate Medical Federation, University of London, Maudsley Hospital, London, S.E.* 5)

A recent survey of respiration in cerebral systems (McIlwain, 1955) showed known rates of oxidation of reduced nicotinamide nucleotides to be collectively slower than the maximal rate of respiration of the brain or of separated cerebral tissues. The rates have accordingly been re-examined.

Optimal conditions for measuring cytochrome *c* reductases, or the oxidation of reduced cozymase, have been found to include histidine or a surrogate. Rates now observed reach 1000 μmoles nucleotide oxidized/g. tissue/hr. They are sensitive to the concentration of reduced cozymase at levels up to 150 μM. As the reduced cozymase levels in rat brain (Glock & McLean, 1955) correspond to 130 ± 55 μmoles/g., control of respiration in part through the level of reduced coenzymes is feasible.

Reduced 2′-phosphocozymase was relatively slowly oxidized in the presence of cerebral tissues and cytochrome *c* or oxygen, but much more rapidly with glutathione (Rall & Lehninger, 1952). Rates up to 200 μmoles nucleotide/g. tissue/hr. have now been observed, again considerably above previous observations. Reduced cozymase was oxidized only slowly in this system. Other indications of the distinctness of systems metabolizing the two coenzymes have been obtained.

The rates now observed are collectively much greater than the tissues' maximal rate of respiration, which is about 300 μatom O or μmoles reduced nucleotide/g. tissue/hr. The rates of oxidation of reduced cozymase are not uniform in different parts of the adult brain; they are not highest in the parts of greatest respiratory rate in the adult animal but, curiously, in those of greatest rate at an earlier stage of development.

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A Benzoic Acid Adsorption Method for Extracting Parathyroid Hormone from Human Urine. By BERYL M. A. DAVIES and RUSSELL FRASER. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Evidence that parathyroid hormone (P.T.H.) can be excreted in mammalian urine was obtained from experiments with mice injected with 'Parathormone' (Eli Lilly Co.) labelled with ^{131}I by a modification of Talmage, Dixon, Bukantz & Dammin's (1951) method.

Attempts were therefore made to extract the hormone quantitatively from human urine. 'Parathormone', or ^{131}I-labelled 'Parathormone', was added to urine, after the destruction of endogenous hormone by HCl. After extraction, hormone recovery was tested either by a mouse urine

phosphate assay (Davies, Gordon & Mussett, 1955) or by radioactivity measurements, assuming that high ^{131}I recovery indicated high hormonal recovery.

Three extraction procedures, ultrafiltration and adsorption on aluminium hydroxide or benzoic acid, were investigated and only the latter led to a satisfactory method. In this, 48 hr. urine, refrigerated as passed, was adjusted to pH 3·5. At 0°, 10 ml. of saturated benzoic acid in absolute ethanol were added per 100 ml. urine (except in diuresis, when proportionately less benzoic acid was used). The precipitate formed (precipitate A) was dried. Originally, the benzoic acid was dissolved by three washings with absolute ethanol (2·7 l.) at 0°. The residue (precipitate B), containing hormone, was dried. Bioassay and radioactivity measurements showed that about 80% of the added hormone was recovered. P.T.H. activity is not lost by boiling at an acid pH: neither was that of precipitate B.

Assuming 'Parathormone' to contain 100 phosphate units/ml., human urinary excretions of P.T.H. (units/24 hr.) were:

Normal subjects (4): average 67, range 61–71.

Confirmed hyperparathyroid patient (uraemic): 139.

Re-extraction of above hyperparathyroid urine: *ca.* 16.

Hypoparathyroid patient: <30.

For six normal subjects, the average weight of precipitate B from 48 hr. urines was 1·0 g. Biuret estimations showed the average protein content to be 0·59 g.; part of the residue was benzoic acid. Attrition of precipitate A and five ethanol washings reduced the weight of precipitate B in two experiments to 0·3 g. On bioassay, parathyroid activity was similar to that obtained in the original procedure. Further purification of precipitate B is being attempted in order to increase the solubility of this product and so facilitate animal testing.

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The Fluorimetric Estimation of Adrenaline, Noradrenaline and Hydroxytyramine in Urine.

By H. Weil-Malherbe. (*Runwell Hospital, Wickford, Essex*)

If the methods of Weil-Malherbe & Bone (1952, 1953) and Lund (1949, 1950) for the fluorimetric estimation of adrenaline and noradrenaline are compared, it is found that both give approximately the same result when applied to plasma (Weil-Malherbe, 1955), but that the former gives much higher results than the latter with acid-hydrolysed urine. This is due to the presence of hydroxytyramine (von Euler, Hamberg & Hellner, 1951), 3:4-dihydroxyphenylacetic acid (von Euler, von Euler & Floding, 1955) and possibly other acidic catechols in hydrolysed urine. These substances form fluorescent derivatives with ethylenediamine, but do not interfere with the methods based on the formation of 3:5:6-trihydroxyindole (Lund, 1949).

Acidic and basic catechol compounds could be separated by either anion or cation exchange. The basic fraction is obtained in a high degree of purity by adsorption on a cationic resin in the H^+-form (Zeokarb 225, water regain 1·8, 40–70 mesh) and elution with 0·2 M-$BaCl_2$. This resin treatment is preceded by passage of the urine through a column of alumina at pH 8·5 and elution of the catechol fraction with acid.

The purified fraction of catecholamines has been investigated by paper chromatography and bioassay. The three catecholamines were found to be the only constituents giving rise to yellow-green fluorescence with ethylenediamine. It is therefore possible to estimate the sum of the three catecholamines by the ethylenediamine method, using a yellow filter. Adrenaline and noradrenaline are estimated separately by the method of Lund (1949, 1950) or of von Euler & Floding (1955). Hydroxytyramine is obtained by difference.

Recoveries of added catecholamines varied from 60–110%, mean 87%. The concentrations found in 30 samples of acid-hydrolysed urine were (means $\pm$ S.E.): adrenaline $11·2 \pm 0·89$, noradrenaline $24·8 \pm 1·68$, hydroxytyramine $82·3 \pm 13·3$ μg./l.

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Some Aspects of the Reactions of D-Glucose with Glutamic Acid, Valine and Lysine. By S. LEWIN and C. FOX. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The reactions of glucose with valine, lysine and glutamic acid have been noted (Agren, 1940; Kubota, 1941), but their mechanism has apparently not been investigated. The 'equilibrium' constant of the valine–glucose interaction at 30° was given as 5·5 (Katchalsky & Sharon, 1953) on the assumption that the reaction involves one molecule of glucose and one molecule of the valine anion. Preliminary investigation of the glutamic acid–glucose reaction (S. Lewin & D. A. T. Southgate, unpublished observations) showed, however, that the monoglucose mechanism cannot explain the data obtained.

We have examined these reactions systematically, aerobically and anaerobically, using varying amino acid concentrations (0·1 to 0·0143M), initial pH values (7–9), glucose concentrations (up to 1·2M) and temperatures (20–50°), using techniques and apparatus (Lewin, 1953, 1955*a*) similar to those reported in the study of histidine–glucose interaction (Lewin, 1955*b*, 1956).

The phenomenon of consecutive steady pH states originally observed in the histidine–glucose reaction was encountered in all the three amino acid–glucose interactions. The results obtained in the first steady pH state require the recognition of the coexistence of both monoglucose and diglucose reversible interactions with one molecule of the amino acid anion involved. The overall three reactions, in the first steady pH state, were found to be exothermic. These results are parallel to those obtained in the alanine–glucose and glycine–glucose interactions (Lewin & Kosinski, 1956).

The respective aerobic pH depression values were practically the same as those obtained anaerobically at low temperature and low glucose concentrations; but with rise in temperature, glucose concentration and steady pH state number, the aerobic ΔpH values recorded were noticeably higher than the anaerobic values.

Decreasing amino acid concentrations in the glutamic acid–glucose and lysine–glucose interactions, other things being equal, resulted in some increase in the pH depression values. But this phenomenon was noted in the valine–glucose interaction only at 50°.

The addition of urea (up to 2·94M) NaCl and Na_2SO_4 (up to 0·368M) did not affect the pH depression values.

Browning of the solutions was noted in all the cases examined, and the colour intensities developed were parallel to those observed in the histidine–glucose interaction. But the aerobic inhibition of the colour development was far more pronounced, resulting in some cases in total suppression.

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Some Aspects of the Reaction of L-Histidine with D-Ribose, D-Xylose, Fructose, Glucose and Lactose. By S. LEWIN. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The phenomenon of consecutive steady pH states observed originally in histidine–glucose solutions (Lewin, 1955, 1956) and later in lysine–glucose, valine–glucose, glutamic acid–glucose (Lewin & Fox, 1956), glycine–glucose and alanine–glucose solutions (Lewin & Kosinski, 1956) has now been found with other systems such as histidine–ribose, histidine–xylose, histidine–fructose and histidine–lactose.

The times required for the attainment of, and the lifetimes of, the steady pH states were much shorter in the pentose–histidine than in the hexose–histidine interactions. Thus at 20° glucose–histidine solutions required about a week for the attainment of the first steady pH state—which lasted for about 4 weeks—while the corresponding pH state in ribose–histidine solutions was attained in about an hour and lasted only about 3 days; the respective 'equilibrium' constants were, however, not greatly different, e.g. 5·0 for the ribose–histidine and 4·3 for the xylose–histidine compared with 3·9 for the glucose–histidine reaction. The first two values have been obtained over the concentration range up to 0·6M pentose where the results are in agreement with the reversible reaction of one pentose molecule with one molecule of the histidine anion.

The increase in the pH depression values with decrease in amino acid concentration, noted in the last three references, has been investigated further in the histidine–glucose and histidine–fructose reactions, and has been found to be more pronounced, particularly in histidine–fructose solutions, with histidine concentrations below 0·0143M. In some cases the increased effect was so great that no steady pH states could be noted, a continuous increase in the pH depression values being obtained instead.

The development of brown colour, as associated with the 'browning reaction', was observed in all the histidine–monosaccharide solutions investigated. But, under similar conditions, fructose, ribose and xylose developed greater colour intensities than the corresponding glucose solutions. However, in the case of the xylose–histidine interaction a variation was noted in that the initial colour developed was distinctly green at first; gradually, however, the solution browned to such an extent that no green tint was visible.

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The Excretion of Bilirubin as an Ester Glucuronide, Giving the Direct van den Bergh Reaction.

By Barbara H. Billing and G. H. Lathe. (*Research Laboratories, Queen Charlotte's Maternity Hospital, London, W. 6, and the Institute of Obstetrics and Gynaecology, University of London*)

The excretion of bilirubin involves its conversion to an unstable substance (pigment II of Cole, Lathe & Billing, 1954). Unlike bilirubin, it is water soluble, and gives a direct van den Bergh reaction. This transformation has been investigated by comparing the two dipyrryl azopigments (A and B) which are formed on coupling bilirubin and pigment II with diazotized aniline (Billing, 1954). One molecule of bilirubin yields 2 molecules of pigment A (Overbeek, Vink & Deenstra, 1955), while pigment II produces the more polar pigment B.

Fresh human hepatic bile was collected by fistula, and contained mainly pigment II. Diazotized aniline was added. Pigment B was salted out with ammonium sulphate, extracted with methanol, and freed of lipid, pigment A, and conjugated bile acids, by a series of counter-current distributions. It was purified by precipitation from methanol–ethanol (1:5) at $-12°$. Pigments A and B had similar absorption spectra, but on a weight basis B had a smaller extinction. Mild alkaline hydrolysis of pigment B yielded pigment A and a reducing substance, which moved and stained like glucuronic acid when chromatographed on paper. Quantitative analysis (Fishman & Green, 1955) showed that in pigment B the molar proportion of glucuronic acid to pigment A was 1, and together they accounted for 87% of the dry weight of the purest preparation of pigment B.

β-Glucuronidase (mouse liver, locust crop juice) converted pigment B to A. Because of its alkaline lability it seems probable that pigment B is an ester glucuronide of pigment A. Accordingly the parent substance, pigment II, might be either the mono- or di-glucuronide of bilirubin. The glucuronic acid content of bile is usually insufficient to account for the conjugation of both carboxyl groups of all the diazotizable pigments. However, the yield of pigment B from impure pigment II suggests a di-glucuronide structure.

Alkali treatment of hepatic bile changed the usual direct van den Bergh reaction to an indirect one, characteristic of bilirubin. Sera from patients with obstructive jaundice and hepatitis, containing direct reacting pigments, had increased amounts of alkali labile glucuronides.

It seems probable that the main pathway of bilirubin excretion in the human is through conjugation with glucuronic acid.

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Excretion of Cystathionine in Pyridoxine Deficiency. By H. BLASCHKO and D. B. HOPE. (*Department of Pharmacology, University of Oxford*)

In chromatograms of urine from rats reared on a diet deficient in pyridoxine, a ninhydrin-positive spot appears which is not present in the urine of litter-mates receiving adequate vitamin B_6 (Blaschko, Datta & Harris, 1953). This spot was provisionally considered to be phosphorylethanolamine.

The unknown spot has now been identified as L-cystathionine. When phosphorylethanolamine was added to urine, two closely adjoining but distinct spots were seen in the chromatograms, but upon addition of L-cystathionine there was only one spot. The unknown spot gave the test with platinic iodide characteristic of S-compounds (Winegard, Toennies & Block, 1948). In the solvent system described by Hoare & Work (1955) the L and D isomers of cystathionine could be separated; the unknown substance appeared in the position of the L isomer. The unknown compound behaved like cystathionine on treatment with Raney nickel (Berridge, Newton & Abraham, 1952). Professor D. D. Woods kindly investigated the activity of the compound with mutants of *Esch. coli* of the methionine series and found its properties consistent with those of cystathionine.

The mammalian enzyme that splits cystathionine is known to be a pyridoxal protein. Our experiments show that B_6 deficiency causes a metabolic block; cystathionine, normally an intermediary metabolite, appears as an end-product of metabolism.

We are grateful to Dr Datta for the phosphorylethanolamine and to Professor Du Vigneaud for the isomers of cystathionine.

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A Comparison of Manometric Methods of Estimating Hydrogenase with the Deuterium Exchange Method. By T. KAMEYAMA, Y. KONDO and N. TAMIYA. (*Chemistry Department, University of Tokyo, and Biochemistry Department, University of Oxford*)

As shown previously (Tamiya, Kameyama, Kondo & Akabori, 1955), hydrogenase activity in bacteria can be estimated by following manometrically the evolution of hydrogen in the presence of reduced methylviologen. This method differs from the usual method for estimating this enzyme by hydrogen uptake in the presence of a hydrogen acceptor. It appeared worthwhile to compare these different manometric methods with the deuterium exchange method (Hobermann & Rittenberg, 1943), which is believed to measure hydrogenase activity directly. The final method for using methylviologen was as follows: the enzyme preparation, 0·25M phosphate buffer at pH 7·0, and 0·0033M methylviologen were placed in the main compartment and 6 mg. sodium dithionite was placed in the side arm of the Warburg vessel. Alkaline pyrogallol and paper were placed in the centre well; the gas phase was nitrogen. After 10 min. equilibration at 30°, the dithionite was mixed with the enzyme and the hydrogen production followed manometrically. The rate was linear until all the dithionite was oxidized. When the rate of hydrogen production was less than 2000 μl./hr. the rate was proportional to the enzyme concentration. This is in contrast to the methylene blue method where the rate of hydrogen uptake is generally not proportional to the enzyme concentration. The methylviologen method thus shows no dilution effect. The deuterium exchange experiment was carried out in a specially designed flask and HD in the gas phase was estimated in a mass spectrometer. Na dithionite was also added as this stimulated the enzyme reaction (Fisher, Krasna & Rittenberg, 1954). The exchange rate (R_E) was calculated by the method of Farkas & Fisher (1947). The ratio of R_E value to the rate constant of the reaction measured by the methylviologen method showed a constant value of 16 with different enzyme samples from intact cells or sonic extracts of *Proteus vulgaris*, suggesting that both reactions are catalyzed by the same enzyme. The methylviologen method was satisfactory with intact cells and extracts of *Escherichia coli*, *Clostridium pasteurianum*, but not with *C. welchii*, because of the presence of an enzyme decomposing dithionite.

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Further Observations on the Specificity of Uridine-diphosphate-glucuronic Acid as Glucuronyl Donor. By G. J. DUTTON and J. H. SPENCER. (*Department of Biochemistry, Queen's College, Dundee, University of St Andrews*)

Uridine-diphosphate-glucuronic acid (UDPglucuronic acid) (Dutton & Storey, 1953; Storey & Dutton, 1955) is necessary for synthesis, in liver homogenates, of the 'ether'-linked *o*-aminophenyl- and (−)-menthyl-glucuronides (Dutton & Storey, 1951, 1954), and of the 'ester'-linked *o*- and *p*-aminobenzoylglucuronides (Dutton, 1955). Phenolphthalein, thyroxine, certain corticosteroids (Isselbacher & Axelrod, 1955) and other steroids (Storey & Dutton, 1956) have been similarly conjugated, UDPglucuronic acid again acting as glucuronyl donor.

The work reported here suggests that, in addition to these compounds, certain aliphatic alcohols and fatty acids also accept glucuronic acid from the nucleotide, which thus apparently functions as glucuronyl donor to a wide range of aglycones (ROH) whose only structural requirement would seem to be an hydroxyl group:

ROH + UDPglucuronic acid →

R-O-glucuronic acid + UDP.

The enzyme system responsible is in each case located in the small particulate, or microsomal, fraction of the homogenate (Dutton & Storey, 1954; Strominger, Kalckar, Axelrod & Maxwell, 1954).

α-Ethylhexanoic acid and *tert.*-amyl alcohol were selected from those aliphatic acids and alcohols known to be excreted as glucuronides (Kamil, Smith & Williams, 1953*a*, *b*). Presence of UDPglucuronic acid and $1{\cdot}38 \times 10^{-3}$M α-ethylhexanoic acid during incubation was necessary for formation of an ethyl acetate-extractable compound giving a positive naphthoresorcinol reaction. β-Glucuronidase treatment destroyed this compound; such destruction was strongly inhibited by boiled saccharate solution (see Levvy, 1952). Thus the compound appeared to be a glucuronide (see Dutton, 1955). Known 2-ethylhexanoylglucuronide behaved likewise.

The compound was hydrolysed with acid (Kamil, Smith & Williams, 1953*b*), the aglycone extracted with ether and chromatographed beside known α-ethylhexanoic and related acids; it travelled like known α-ethylhexanoic acid.

tert.-Amyl alcohol glucuronide formation was indicated similarly, with substrate molarity of $1{\cdot}42 \times 10^{-2}$, conjugate extraction with ethanol-ether mixture from acidified $(NH_4)_2SO_4$ solution, and aglycone chromatography (after enzymic hydrolysis of the conjugate) utilizing xanthogenate formation (Kariyone & Hashimoto, 1951). The supposedly pure glucuronide behaved comparably.

Competitive inhibition occurred when either substrate was added to the *o*-aminophenylglucuronide-synthesizing medium (Dutton & Storey, 1954), suggesting that the same enzyme system might be involved. pH and oxygen requirements were those found for other substrates, and again synthetic activity was located in the microsomes.

'Crude factor' was used in preliminary studies, confirmation being obtained with a pure preparation (Storey & Dutton, 1955).

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Glucose Catabolism by Strains of *Trypanosoma rhodesiense*. By P. T. GRANT and J. D. FULTON. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The blood-stream form of the pathogenic African trypanosome, *Trypanosoma rhodesiense*, utilizes glucose at a high rate when incubated at 37° in a phosphate-buffered saline medium (pH 7·4) containing 0·2% (w/v) glucose and 10% (v/v) rat serum. The products of this catabolism have been identified as ethanol, formate, acetate, pyruvate, succinate and glycerol (Fulton & Stevens, 1945), and it has now been found that 80% of [U-^{14}C]-glucose utilized by the parasite can be accounted for as extracellular pyruvate, 0·93% being evolved as $^{14}CO_2$. The radioactivity in the respiratory CO_2

and pyruvate obtained in similar 2 hr. incubations containing either [U-^{14}C]-, [3,4-^{14}C]-, or [1-^{14}C]-glucose and the distribution of isotope in the pyruvate samples was consistent with the view that glucose is catabolized via the classical glycolytic pathway (the Embden–Meyerhof–Parnas scheme). Essentially similar results were obtained with drug-resistant (stilbamidine-fast and atoxyl-fast) strains of the same trypanosome.

Pyruvate is thus the main end-product of glucose catabolism by the parasite under *in vitro* conditions, and neither coenzyme A, diphosphopyridine nucleotide (DPN^+ or DPNH), thioctic acid, cocarboxylase and yeast extract added singly or together to the incubation medium, nor substrates such as oxaloacetate, citrate and CO_2, had any marked effect on the amounts of pyruvate and respiratory CO_2 produced. Moreover, it seems likely that pyruvate is also the main end-product of glucose catabolism of the growing trypanosome *in vivo*, since the blood-pyruvate levels of rats become enormously enhanced during the later stages of an infection.

At the end of a 2 hr. incubation period with [U-^{14}C]glucose, 1·73% of the radioactivity in the glucose utilized had been incorporated into the cellular material of the parasite. 0·9% was present as fat and was almost entirely associated with glyceride-glycerol; fatty acids contained only traces of radioactivity. In the trypanosome protein (containing 0·64% of the radioactivity of the glucose utilized), only aspartic acid, alanine, glycine and serine contained ^{14}C. For these reasons, the lack of appreciable radioactivity in fatty acids and protein-bound glutamic acid would indicate that certain reactions of the tricarboxylic acid cycle do not take place to a significant extent under *in vitro* conditions.

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The Metabolism of Ethyl Mercaptan. By G. A. Snow. (*Imperial Chemical (Pharmaceuticals) Limited, Hexagon House, Blackley, Manchester*, 9)

It has been found independently by several groups of workers (Del Pianto, 1953; Brown *et al.* 1954; Kushner *et al.* 1955; Davies *et al.* 1956) that ethyl mercaptan and related compounds exhibit a remarkable antitubercular activity in experimental animals. This specific activity occurs only *in vivo*; when tested *in vitro*, ethyl mercaptan shows only the feeble inhibitory activity common to other alkyl thiols. Interest has therefore centred on the metabolism of ethyl mercaptan.

Diethyl disulphide and ethyl thiolbenzoate labelled with ^{35}S were prepared and administered to mice and guinea pigs. Examination of the tissues showed that radioactivity was quickly and uniformly distributed throughout the body with no observable tendency to concentrate in a particular organ or tissue. Some radioactivity (10–15% of the dose) appeared in the breath, little in the faeces, but most was excreted in the urine. Urinary excretion was exponential, 50% of the total recoverable radioactivity appearing in the first 10 hr. In both mouse and guinea pig about 85% of the urinary radioactivity was due to sulphate, but the remainder was largely represented as a single radioactive spot on paper chromatography. An identical spot, but without any indication of sulphate, was given by extracts of tissues from dosed mice. From experiments with radioactive guinea-pig urine the organic metabolite appeared to be neutral, chemically stable and somewhat volatile.

Urine from guinea pigs dosed with unlabelled diethyl disulphide was extracted with chloroform, ether-soluble material was removed, and the resultant product distilled. The main fraction boiling at 60–70°/0·05 mm. was recrystallized from ether to give long needles (m.p. 33·5°) of ethyl methyl sulphone. The identity of this compound with the metabolite was checked by comparing ethyl methyl [^{35}S]sulphone with concentrates from the urine of animals dosed with $(C_2H_5{}^{35}S)_2$. Indistinguishable R_F values were observed on paper chromatograms run with three different solvents. Counter-current distribution of the urine concentrate using a chloroform–water system confirmed the sulphone as the main metabolite but also showed the presence of another which had not been revealed by paper chromatography. The constitution of the second metabolite is undetermined. Neither metabolite possesses significant antitubercular activity. The biochemical significance of the metabolism of ethyl mercaptan will be discussed.

The author thanks Dr A. R. Martin for dosing and care of animals.

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The Isolation of Further Vitamin B_{12} Analogues. By K. H. Fantes and Cynthia H. O'Callaghan. (*Glaxo Laboratories Ltd., Sefton Park, Stoke Poges, Bucks.*)

Ford, Holdsworth & Kon (1954, 1955) have shown that the vitamin B_{12}-requiring *Escherichia coli* mutant was capable of producing vitamin B_{12} analogues from factor B and certain benziminazoles or purines. We isolated an analogue from the metabolite fluid after a fermentation by *S. griseus* in the presence of *o*-phenylenediamine, but without factor B (Fantes & O'Callaghan, 1954, 1955). Further analogues were prepared by this method.

The addition of graded levels of 1:2-diamino-4:5-dichlorobenzene did not lead to an appreciable production of the 5:6-dichlorobenziminazole analogue, though this was formed readily when 5:6-dichlorobenziminazole itself was used. 20 μg./ml. of the latter completely suppressed the production of vitamin B_{12} so that only the new analogue was formed, whereas mainly vitamin B_{12} was formed in the presence of the diamine (even at 80 μg./ml.). The chlorine-containing analogue was isolated by methods previously described (Fantes & O'Callaghan, 1955). In a similar fashion crystalline vitamin B_{12} analogues containing 2:3-naphthiminazole and 5-aminobenziminazole were isolated after fermentations in presence of 80 and 250 μg./ml. of the respective precursors. The addition of 0·05 % sodium hydrosulphite to the medium helped to produce the aminobenziminazole compound. From the same fermentation broth another crystalline factor was obtained, probably the 5-hydroxybenziminazole analogue (B_{12}-Factor III, Friedrich & Bernhauer, 1955), since it appeared to be identical with the product obtained from nitrous acid treatment of the 5-aminobenziminazole analogue.

5:6-Dichlorobenziminazole, 2:3-naphthiminazole and 5-aminobenziminazole were all identified in the acid hydrolysates of the respective B_{12} factors by their R_F values and u.v. absorptions; the aminobenziminazole also gave a pink colour on coupling with β-naphthol. The constitution of the chlorine-containing analogue was confirmed by X-ray crystallographic evidence (Kamper & Hodgkin, 1955).

A number of other micro-organisms have been found to be able to form analogues, but most of them seem unable to incorporate benziminazoles substituted in positions 2 or 4, or purines. Certain Clostridia, such as *C. tetani* and *C. welchii*, are able to form analogues containing purines, 4-substituted benziminazoles or triazoles.

The two analogues so far tested (containing 5:6-dichlorobenziminazole and 2:3-naphthiminazole) have been found active in the treatment of pernicious anaemia patients. The clinical trials were kindly carried out by Professor G. M. Wilson, Department of Pharmacology and Therapeutics, at the University of Sheffield.

Other biological tests are in progress.

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Chromogenic and Fluorogenic Reactions of Steroids in Concentrated Acids. By H. Kalant* (introduced by F. G. Young). (*Department of Pathological Chemistry, University of Toronto, Canada*)

Treatment with concentrated sulphuric, phosphoric or other acids has been used for identification and measurement of many steroids, by absorption spectrophotometry or fluorophotometry of the reaction mixtures. Attempts to correlate reactivity with the number of hydroxyl groups or other substituents in the steroids (Goldzieher, Bodenchuk & Nolan, 1954; Linford & Paulson, 1952) have yielded no satisfactory explanation of the mode of action of concentrated acids on steroids.

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In the present work, absorption spectra of various adrenocortical, androgenic, oestrogenic, and other steroids were compared after treatment with concentrated H_2SO_4 (Zaffaroni, 1953) and with 99–100% H_3PO_4 (Kalant, 1954). Agreement between the two spectra varied from excellent with hydrocortisone to almost none with oestriol. Spectra in H_3PO_4 were stable for at least 24 hr. Those in H_2SO_4 underwent changes with time, or on heating, which increased considerably their resemblance to the H_3PO_4 spectra. Progressive dilution of both sets of reaction mixtures produced striking sequences of change in absorption patterns, which were essentially similar in both acids for a given steroid. In general, corresponding spectra were found at lower concentrations of H_2SO_4 than of H_3PO_4.

Examination of the reaction mixtures at each stage of dilution, under different visible and ultraviolet illuminations, showed the same changes in visible fluorescence in both sets. Each steroid behaved in a different manner. For example, cortisone in concentrated acid showed blue fluorescence under 365 mμ. illumination, which decreased progressively on dilution. In contrast, hydrocortisone in concentrated acid showed pale blue-white fluorescence under 365 mμ. and bright yellow-green under 436 mμ.; on dilution, fluorescence under 436 mμ. disappeared, but a brilliant green appeared under 365 mμ. Examination of the fluorescences by spectrofluorometry (Gornall & Kalant, 1955) and by spectrography showed each to be a complex emission of two or more bands which altered in relative intensity on dilution.

Ethylene chloride extracts of H_2SO_4 reaction mixtures, after either neutralization or extreme dilution, were chromatographed on paper. Individual fractions were eluted, redissolved in concentrated acid, and their absorption and fluorescence properties studied, including the changes on redilution.

All the findings suggest a general reaction pattern involving formation of an unstable intermediate which undergoes degradation into several different stable products, capable of reversible associations in acid. No evidence was found for the polymerization hypothesis of Jaffe (1952).

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Carbamyl Phosphate-Aspartate Transcarbamylase. By J. M. LOWENSTEIN* and P. P. COHEN.
(*Department of Physiological Chemistry, University of Wisconsin, Madison, U.S.A.*)

It has been shown previously that carbamyl aspartate (CA) is formed enzymically from aspartate and an intermediate compound which acts as carbamyl group donor (Lowenstein & Cohen, 1954, 1955). Similar conclusions were reached by Reichard & Hanshoff (1955). The identification and synthesis of carbamyl phosphate (Jones, Spector & Lipmann, 1955) has greatly facilitated the study of CA biosynthesis.

The enzyme ('carbamyl phosphate-aspartate transcarbamylase') which forms CA requires only L-aspartate and carbamyl phosphate. CA formation has been assayed by a method which makes use of the fact that the ^{14}C-carbamyl carbon of carbamyl phosphate, but not that of CA, is expelled as $^{14}CO_2$ when heated with dilute HCl. The enzyme occurs predominantly in the supernatant obtained after high speed centrifugation of rat-liver homogenates. It has an optimum pH of 9·2, and is inhibited 50% by 5×10^{-3}M iodoacetate, 70% by 1×10^{-3}M *p*-chloromercuribenzoate, and 70% by 1×10^{-1}M arsenate. The enzyme has been found to be present in the following rat tissues: brain, gastric mucosa, intestinal mucosa, kidney, liver, lung, leg muscle, pancreas, spleen and testis. Its presence in rat blood and heart is doubtful. It occurs also in pigeon tissues, a finding which suggests that carbamyl phosphate is synthesized in birds. The specificity of the carbamyl phosphate transcarbamylase reaction has been studied in supernatants obtained by low speed centrifugation of rat-liver homogenates. Out of thirty amino acids tested, only ornithine and aspartate react readily as acceptors of the carbamyl group. Very low rates of reaction are also observed with asparagine, *iso*-asparagine and lysine.

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Species Differences in the *o*- and *p*-Hydroxylation of Aniline. By D. V. PARKE and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

Some carcinogenic aromatic amines appear to produce their biological effects after conversion *in vivo* to *o*-aminophenols. In the case of 2-naphthylamine there appears to be a correlation between the amounts of 2-amino-1-naphthol conjugates in the urine and the incidence of bladder tumours in different species, and this provides a possible explanation of the differing susceptibilities of various species to the action of the carcinogen (Bonser, Clayson & Jull, 1951).

During an investigation of the metabolism of ^{14}C-aniline it became possible, by the isotope dilution technique, to measure the extent of *o*- and *p*-hydroxylation in various species. The urine of animals given 250 mg./kg. of aniline was collected for 48 hr. and the total amounts of *o*- and *p*-aminophenol excreted were determined. The average *para*/*ortho* ratio for two animals of each of the species so far investigated were: gerbil, 15; guinea pig, 11; hamster, 10; rabbit, 6; rat, 5; mouse, 3; ferret, 1; cat, 0·4. The total amino phenols excreted in this time was 40–60 % of the dose. It is to be noted that the ferret and cat produce much more *o*-isomer than the others.

One possible explanation of these results is that two enzymes are involved, an '*ortho*-hydroxylase' and a '*para*-hydroxylase', and that these enzymes occur in different proportions in different species. This possibility is now being investigated. Another possibility is that some animals can acetylate the aromatic amino group more extensively than others and consequently *ortho*-hydroxylation is sterically hindered. However, it is known that the cat can acetylate aromatic amines and therefore this explanation seems unlikely. Other animals (including the dog which does not acetylate aromatic amines, cf. Marshall, 1954) are being studied.

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DEMONSTRATIONS

Total Body Water in Obesity. By G. L. S. PAWAN. (*Professorial Medical Unit, The Middlesex Hospital, London, W.* 1)

Total body water was estimated by the urea method of McCance & Widdowson (1951) with precautions described by Pawan (1954).

In 18 apparently normal non-obese subjects (10 males, 8 females, aged 18–54 years), the urea space ranged from 58 to 66 (mean 62) % of the body weight. In a series of 34 obese patients (4 males, 30 females, aged 16–66 years), 28–320 (mean 63) % above ideal body weight for age, sex and height, the urea space was found to be 25–58 (mean 46) % of body weight. Total body water expressed as a percentage of body weight was found to vary inversely with the degree of adiposity.

When weight reduction (mean 10·2 kg.) was induced in the obese series of patients by calorie restriction, the mean absolute value for total body water decreased by 1·9 l., but expressed as a percentage of body weight, the value increased from 46 to 49 % of body weight.

Reasonably good agreement was found between the urea, antipyrine (Soberman *et al.* 1949), and *N*-acetyl-4-amino-antipyrine (NAAP) (Brodie *et al.* 1951) spaces. In 7 obese subjects, the mean value for the antipyrine space was 4·9 % less than the mean NAAP space. The mean urea space was 2·8 % greater than the antipyrine space.

I thank Professor Alan Kekwick for permission to investigate the patients reported.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **350th Meeting** *of the Biochemical Society was held in the Department of Biochemistry of the University of Glasgow on 13th April* **1956**, *at* **11.30** *a.m., when the following papers were read:*

COMMUNICATIONS

The Effect of Iron Status on the Pattern of Free Amino Acids and Organic Acids in Plant Leaves.

By P. C. DE KOCK and R. I. MORRISON. (*The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen*)

The amino and organic acids of leaves of plants of differing iron status have been studied by chromatographic methods. The leaves were macerated in a Waring Blendor in 80% acetone (HCl acidified), and the extract passed over Zeo-Karb 225 and IR-4b resins after evaporation of the acetone and other procedures. The amino acids in the eluate from the Zeo-Karb were studied semi-quantitatively by 2-dimensional paper chromatography (phenol–ammonia and butanol–acetic acid–water). Organic acids were quantitatively estimated on a silica gel column using gradient elution with *tert.*-amyl alcohol–chloroform mixtures. Inorganic analyses for iron, phosphorus, calcium and potassium were conducted by methods current at this institute.

All iron-deficient plants were found to contain larger amounts of free amino acids in both roots and leaves than did healthy green plants. Iron-toxic leaves on the other hand, even when showing chlorosis, had smaller quantities of free amino acids than the healthy leaves.

The total extractable organic acids were found to increase with increasing iron status, thus showing an inverse relationship to the amino acids. Also, iron-deficient plants were seen to contain relatively large amounts of the tricarboxylic acids whereas iron-toxic plants contained more of the dicarboxylic acids (malic, malonic). From the result it appears that the Krebs cycle is operative in plants and that the relative amounts of acids present in the leaves is dependent on the iron status, as measured by the phosphorus-iron ratio. It was thus found that mustard plants poisoned with fluoroacetic acid, an inhibitor of aconitase, showed symptoms of typical iron deficiency with high phosphorus-iron ratio, increased amounts of free amino acids and altered organic acid pattern.

Faecal Excretion in Man after the Administration of Mixed Plant Sterols (Sitosterols).

By SHEENAH K. BISSET and R. P. COOK. (*Department of Biochemistry, Queen's College, Dundee (University of St Andrews)*)

The observations of Peterson and his colleagues (e.g. Peterson, Nichols & Schneour, 1952) that sitosterols hinder the absorption of cholesterol in experimental animals has been extended by various workers to the effect on cholesterol absorption in man (e.g. Pollak, 1953; Joyner & Kuo, 1955). It has been shown that in the blood plasma the level of cholesterol and of lipoproteins is lowered. The experiments to be described were made in an attempt to ascertain the mode of action of vegetable sterols.

Three subjects were fed 10 g. per day of mixed sitosterols for 5 days and the faeces collected before, during and after administration. An ethereal extract was analysed for total lipid and for free and total sterol. The weight of faeces and the amount of lipid (30–40% of the dry wt.) were increased after sitosterol administration. Total sterol increased in amount, but on balance was equal to only about 60% of that fed. There was an increase in the ester sterol excreted, values of from 1 to 1·5 g. per day being found.

The lipids were separated by chromatography on alumina and a fraction rich in sitosteryl esters obtained. It is possible that one mechanism of action of the sitosterol is that it competitively inhibits the esterification of cholesterol in the gut. There is evidence also that 'mixed' crystals of the free sterols are formed. The conversion of sitosterol to non-digitonin precipitable material is still unexplained.

The feeding experiments and collection of faeces were carried out under the supervision of Drs M. F. Oliver and G. S. Boyd, Edinburgh. We are grateful to Dr Max Tishler, Merck and Co. Inc. for a generous gift of sitosterols. One of us (S.K.B.) was in receipt of a personal grant from the Scottish Hospitals Research Trust.

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The Hydrolysis of Glucose 1-phosphate by Rat Liver Extracts. By G. A. J. Goodlad* and G. T. Mills. (*Biochemistry Department, University of Glasgow*)

It was suggested by Broh-Kahn & Mirsky (1948) that no specific glucose 1-phosphatase activity exists in liver and that glucose 1-phosphate (G 1-P) is converted to glucose 6-phosphate (G 6-P) before hydrolysis. Swanson (1950) partially purified a specific glucose 6-phosphatase from liver which had no activity towards G 1-P. Faulkner (1955), on the other hand, has demonstrated the presence in silkworm blood of a phosphatase specific towards G 1-P. As part of a study of the specificity of rat liver phosphomonoesterases we have examined the mechanism of hydrolysis of G 1-P by rat liver extracts.

The hydrolysis of G 1-P by freshly prepared liver homogenates occurred at a higher pH optimum and was more strongly activated by magnesium ions than the hydrolysis of G 6-P. Substrate inhibition was absent when G 6-P was being hydrolysed but was very pronounced with G 1-P as substrate. Rat liver phosphoglucomutase was found to have a pH optimum similar to that for the hydrolysis of G 1-P, to require magnesium ions for activity and to be inhibited by high concentrations of G 1-P. These findings are in support of the views of Broh-Kahn & Mirsky as they are most readily explained by G 1-P undergoing an initial rate controlling mutation to G 6-P.

The intracellular distribution of glucose 1-phosphatase activity was studied. When the different fractions, obtained by differential centrifugation, were assayed singly, negligible activities were obtained. On assaying combined microsomal and non-particulate fractions, however, 85% of the activity of the unfractionated cytoplasm was obtained. Boiled microsomal fraction added to unboiled non-particulate fraction and vice versa failed to show this effect. These results again indicate that the hydrolysis of G 1-P is a two-stage process involving phosphoglucomutase, which occurs only in the non-particulate fraction (Hers, Berthet, Berthet & de Duve, 1951) and glucose 6-phosphatase which was found to be present mainly in the microsomes and to only a very small extent in the non-particulate fraction in these preparations.

No evidence in support of any secondary mechanism for the hydrolysis of G 1-P was obtained in these studies. At pH 5·5, the optimal pH of Faulkner's glucose 1-phosphatase, the rate of hydrolysis by liver homogenates was negligible.

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The Multiple Nature of Rat Liver Acid Phosphatase Activity. By G. A. J. Goodlad* and G. T. Mills. (*Biochemistry Department, University of Glasgow*)

Studies of the effect of magnesium ions on the pH-activity curves of rat liver acid phosphatase activity using various substrates have indicated the existence of a Mg^{2+}-sensitive enzyme (I), optimally active around pH 3·75, and a Mg^{2+}-insensitive enzyme (II), optimally active at pH 5·5. When β-glycerophosphate and adenosine 3′-phosphate were used as substrate, evidence was obtained for the presence of a third enzyme optimally active about pH 6·5.

The shape of pH-activity curves obtained using veronal-acetate buffers differed from those obtained using acetate buffers. Veronal appeared to activate I and inhibit II. Copper ions had no effect on I but inhibited II completely, even at concentrations of 2×10^{-4} M. I was inhibited by citrate and tartrate to a much greater extent than was II.

A partial separation of I and II was achieved by fractionation with acetone in the presence of zinc ions followed by electrophoresis in the Tiselius apparatus.

No difference in the intracellular distribution of the three enzymes was found.

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Cytidine Diphosphate Glycerol and Related Compounds from *Lactobacillus arabinosus*. By J. BADDILEY, J. G. BUCHANAN, B. CARSS, A. P. MATHIAS and A. R. SANDERSON. (*Department of Chemistry, University of Durham, King's College, Newcastle upon Tyne*)

By the use of gradient elution chromatography on Dowex-2 with formic acid, followed by charcoal adsorption and elution, it has been possible to obtain pure samples of the two cytidine nucleotides previously isolated from *Lactobacillus arabinosus* (Baddiley & Mathias, 1954). One of these has now been identified as cytidine diphosphate glycerol (CDP-glycerol). Analysis indicates the presence of two phosphate groups to one cytidine residue. On hydrolysis in N-hydrochloric acid at 100° for 20 min. cytidine 5′-phosphate and two organic phosphates are produced. These organic phosphates have the same R_F values in several solvents as a mixture of α- and β-glyceryl phosphates, the α-form being present in greater amount. The glyceryl phosphates are readily separated on paper, using a *tert.*-butanol–picric acid–water solvent (Hanes & Isherwood, 1949). Only the α-form gave a reaction typical for a 1:2-diol when the paper was sprayed with periodate followed by Schiff's reagent (Buchanan, Dekker & Long, 1950). Confirmation of the identity of the α-phosphate was obtained by oxidation with periodate to glycollic aldehyde phosphate which had the same R_F as the periodate product from authentic α-glyceryl phosphate. The mixture of α- and β-phosphates was dephosphorylated enzymically (polidase) giving glycerol and orthophosphate.

When the nucleotide was treated with phosphatases obtained from the venom of *Crotalus atrox* the products were cytidine, inorganic phosphate and α-glyceryl phosphate. It was shown by paper chromatography that no β-glyceryl phosphate was present. This indicates that the original nucleotide contained an α-glyceryl phosphoryl residue.

The presence of a pyrophosphate linkage between the 5′-position in the cytidine and the α-position in the glycerol, already strongly suggested from the above evidence, was confirmed by alkaline hydrolysis. When heated for 20 min. in dilute ammonia the original substance was partly converted into cytidine 5′-phosphate and glyceryl-1:2 hydrogen phosphate. The identity of this cyclic phosphate was established by comparison on paper chromatography and electrophoresis with an authentic sample.

The second cytidine nucleotide is also a derivative of cytidine 5′-pyrophosphate bearing a substituent on the terminal phosphate group. The nature of this substituent has not yet been fully established, but from the reactions of this nucleotide it appears to be closely related to CDP-glycerol.

We thank the Nuffield Foundation for financial support, and Dr F. C. Belton, Ministry of Supply, Microbiological Research Department, for assistance in the growth of *L. arabinosus*.

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Studies on the Reduction of Nitrate in the Artificial Rumen in the Presence of Various Substrates. By A. J. G. BARNETT and I. B. R. BOWMAN. (*Division of Agricultural Biochemistry, Department of Biological Chemistry, University of Aberdeen*)

The condition of 'oat-hay' poisoning in cattle has been investigated by a number of workers, including Bradley, Eppson & Beath (1939), and is related to high concentrations of nitrate in the feed. These may reach a level of 7% (as KNO_3 on the D.M.) in oat straw (Sjollema, 1931). Nitrate is reduced, by microbial action, to nitrite in the rumen with a resultant toxic effect manifested partly by the occurrence of methaemoglobinuria.

Studies of the reactions involved have been made by Sapiro, Hoflund, Clark & Quin (1949) using rumen liquor, which was incubated without regard to sustaining pH at normal rumen levels, and by Lewis (1951*a*) who performed his experiments on a sheep which had not been fed for 16 hr. This latter worker (1951*b*) established, from *in vitro* studies, that various substances normally present in crops could act as hydrogen donors in the reduction.

In the present work, six artificial rumens (Burroughs, Headley, Bethke & Gerlaugh, 1950) were set up, each containing a rumen liquor inoculum and a mineral salt medium. Two rumens acted as controls, to the third was added cellulose, to the fourth, glucose, to the fifth, dried grass and to the sixth, water-soluble grass pigments. CO_2 was passed through the rumens individually, and they were kept at 39° for 35 hr.; 5 hr. after the start of the run, equal volumes of a solution of KNO_3 were added to each. The emergent gases

were tested for ammonia and nitric oxide but neither were detected, probably as a result of controlling the pH of the medium at pH 6·5–6·7. Nitrate, nitrite, ammonia and protein N were determined on samples collected at intervals, and it was found that nitrite formation was most vigorous when dried grass or grass pigments (containing potential hydrogen donors) were present. Cellulose decreased the apparent nitrite production beneath that attained in the controls but no nitrite was detectable in the rumen containing glucose although the nitrate disappeared. Small amounts of ammonia were produced in all cases while the total recovery of NO_3-N, NO_2-N and NH_3-N was good in all cases. The presence of nitrate and nitrite did not appear to have a completely inhibitory effect on protein synthesis.

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Yeast Branching Enzyme. By D. J. Manners and Khin Maung. (*Department of Chemistry, University of Edinburgh*)

During the purification of phosphorylase and isoamylase from brewer's yeast (Manners & Khin Maung, 1955) we observed that certain protein fractions catalysed a rapid decrease in the iodine staining power of amylose. Further investigation has shown that this activity was caused by a branching enzyme, i.e. an amylose → glycogen transglucosidase.

By ethanol-citrate fractionation (at $-5°$) of a brewer's yeast extract, we have obtained a protein preparation which reduced the iodine staining power of amylose, soluble starch, amylopectin, and amylopectin β-dextrin without concomitant liberation of reducing groups. On incubation of amylose (D.V. 1·33; β-amylolysis limit 84%) with branching enzyme, the iodine absorption value at 680 mμ. decreased by 66 and 81% within 1 and 4 hr. respectively; the β-amylolysis limit likewise decreased, to 50 and 48%. After 20 hr. incubation, the end-product stained red-brown with iodine, the absorption spectrum having a maximum at 520 mμ. (cf. amylopectin $\lambda_{max.}$ 540 mμ., glycogen 460–490 mμ.). Yeast branching enzyme has no action on glycogen or glycogen β-dextrin.

Enzyme action is inhibited by mercuric chloride (5×10^{-4}M); it is optimum at pH 7·0 and 20°, these conditions being similar to those for the branching enzymes (Q-enzymes) of plants (Hobson, Whelan & Peat, 1950). Unlike the branching enzyme from *Polytomella coeca* (Barker, Bebbington & Bourne, 1953), the yeast enzyme is not activated by maltosaccharides.

The branch points synthesized by yeast branching enzyme are hydrolysed by yeast isoamylase, and are therefore similar to those in amylopectin and glycogen (i.e. α-1:6-glucosidic). Treatment of amylose with branching enzyme for 4·5 hr. gave a branched polysaccharide with a β-amylolysis limit of 45%. After treatment with isoamylase, the β-amylolysis limit was 65% (cf. Manners & Khin Maung, 1955).

We are grateful to Professor E. L. Hirst, F.R.S., for his advice and encouragement.

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The Metabolism of Chick Embryonic Heart and Liver Tissue During Adaptation to Tissue Culture Conditions. By J. Paul and Enid S. Pearson. (*H.E.R.T. Tissue Culture Laboratory, Department of Biochemistry, University of Glasgow*)

Respiration, ^{32}P incorporation, protein, lipid, carbohydrate, RNA and DNA content and the metabolism of glucose, lactate and pyruvate have been studied in fresh 15-day chick embryonic heart and liver and during the 6 days following explantation and maintenance as tissue cultures.

Fresh heart tissue initially has a very low Q_{O_2} under these conditions. After 24 hr., respiration is significantly greater and is depressed by renewal of the medium. Heart explants utilize glucose rapidly, producing large amounts of lactate. Pyruvate accumulates in the medium to a maxi-

mum concentration and may fall later if the medium is not renewed. ^{32}P incorporation into lipid phosphorus, acid-soluble phosphorus, RNA phosphorus and DNA phosphorus decreases rapidly in the first 24 hr. On renewal of the medium incorporation is stimulated and it may exceed the initial level. Nucleic acids in the tissue rapidly decrease in the first two days. Tissue carbohydrate also decreases while other constituents show little change. Histological sections show rapid disappearance of muscle cell nuclei in the centre of the explant, with better survival at the periphery. Fibroblasts proliferate and form a capsule round the explant as well as growing over the surface of the culture vessel.

Fresh liver tissue has a high Q_{O_2}. Lactate disappears from the medium. Glucose also disappears, but the total amount of hexose in the medium increases at first due to the appearance of non-reducing material. After 24 hr. lactate utilization has ceased and it has accumulated to a level higher than that originally present. It continues to accumulate. Respiration has fallen to a low level. It is not stimulated by renewing the medium. As in heart, there is a marked fall in nucleic acids without much alteration in other tissue constituents. The pattern of ^{32}P incorporation is similar to that observed in heart, the fall in activity in the first day being very pronounced. It is stimulated by renewing the medium. Histological sections show a course of events similar to those in heart explants but the necrotic changes are greater.

We are indebted to Professor J. N. Davidson for the provision of facilities, to Miss Ina Withers for technical assistance and to the Scottish Hospitals Endowment Research Trust for financial support in this research.

The Excretion of 5-Hydroxyindoleacetic Acid (5-HIAA) by Normal Human Subjects. By M. Dick and A. Todrick. (*Department of Clinical Research, Crichton Royal, Dumfries*)

The isolation of 5-hydroxytryptamine (5-HT) from mammalian plasma and intestinal mucosa, and its subsequent identification in the human body, has aroused considerable speculation as to its physiological function. Erspamer (1954) holds that urinary 5-HIAA excretion is the best indication of the level of 5-HT metabolism.

The presence of 5-HT has been demonstrated in the central nervous system (Amin, Crawford & Gaddum, 1952), and the observation of Gaddum (1953) that the hallucinogenic drug lysergic acid diethylamide is a powerful inhibitor of the action of 5-HT on smooth muscle indirectly supports the view that 5-HT may influence central nervous function (cf. Pletscher, Shore & Brodie, 1955; Rodnight & McIlwain, 1956).

Despite the interest in 5-HIAA the published data on its 24 hr. urinary excretion in humans is limited.

The method employed for the estimation was a modification of that of Udenfriend, Titus & Weissbach (1955). 5-HIAA was estimated in the 24 hr. urines of twenty males and twenty-one females. The males gave a mean of $4{\cdot}20 \pm 1{\cdot}20$ and the females $3{\cdot}72 \pm 0{\cdot}99$ (S.D.) mg./day. A 't' test indicates that these means are not significantly different ($P = 0{\cdot}2$). The combined results have a mean of $3{\cdot}95 \pm 1{\cdot}11$ mg./day. The observed range is 2·2–7·8, the distribution curve being slightly skewed. These results agree with those of Udenfriend *et al.* (1955), four cases, range 2–8 mg./day; Erspamer (1955), five cases, mean 4·5 mg./day and Snow, Lennard-Jones, Curzon & Stacey (1955) forty cases, mean 2·7 μg./ml. urine.

There does not appear to be any correlation of 5-HIAA excretion with age or weight.

When the results are plotted against urine volume, there is some suggestion of a direct correlation. However, in all cases where individual volumes varied widely on successive days, no concomitant variation in 5-HIAA excretion was noted.

The day-to-day variation in 5-HIAA excretion over a period of about 1 week appears to be fairly small, normally not varying by more than 1 mg./day.

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Changes in Glucose Utilization with the Growth of Tissue Cultures of HeLa Cells. By W. C. FULTON, R. SINCLAIR and I. LESLIE. (*Biochemistry Department, Queen's University, Belfast, Northern Ireland*)

HeLa Cells (a strain of human epidermoid carcinoma) were grown in roller tubes with daily changes of a medium consisting of 5% chick embryo extract, and 20% human serum in modified Connaught medium 858 (Healy, Fisher & Parker, 1955). The glucose (G) utilization, lactic acid (LA) and total keto-acid (KA) production were measured over consecutive periods of 2 days and compared with changes in phospholipid, ribonucleic acid and deoxyribonucleic acid phosphorus (DNA-P). Since the average amount of DNA-P per nucleus of the HeLa cell is constant at 1·36 pg.* (Leslie, Fulton & Sinclair, 1955), the cell number can be estimated from DNA-P determinations.

Previous attempts to correlate glucose utilization with increases in cell material or cell number indicated that there was no direct relationship between these two quantities (Willmer, 1942; Westfall, Evans, Shannon & Earle, 1953). Similarly, with the HeLa cells, the glucose utilization did not run parallel to increases in cell number, since, for example, it increased only by about 10% for a fourfold increase in cell number.

Measurements of lactic acid and total keto-acid production revealed that the proportion of glucose appearing as these compounds diminished with increasing cell number. Presumably, as the glucose concentration of the medium falls, an increasing proportion of the lactic and keto-acid is used for energy production. In support of this view, it was found that there was a linear relationship between the cell number and the glucose utilized but not accounted for as lactic and keto-acid.

While lactic acid production could account for nearly half the glucose utilized (LA/G molar ratio approx. 0·9) in the initial cultures, the total keto-acid represented less than 5% (KA/G molar ratio = 0·05 or less). With increasing cell number, the ratio of lactic acid to keto-acid (LA/KA) tends to fall. Insulin has been found to stimulate the growth of HeLa cell cultures (Leslie *et al.* 1955), but in this case the ratio LA/KA in the medium always rises significantly. From these results it can be concluded that the change in LA/KA under insulin treatment is not simply a consequence of the additional growth.

The authors thank Dr G. W. Sheffield, Department of Bacteriology, University College Hospital Medical School, London, for the cultures of HeLa cells which enabled us to build up our stock.

* pg. = picogram = 1×10^{-12} g.

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The Influence of the Medium on the Insulin Response of Two Unrelated Human Cells Grown in Tissue Culture. By I. LESLIE, W. C. FULTON and R. SINCLAIR. (*Biochemistry Department, Queen's University, Belfast, Northern Ireland*)

Human skin fibroblasts from a 20-week embryo and from a strain of HeLa cells have been grown in roller tubes under closely comparable conditions. Two types of media have been used, a maintenance medium (MM) consisting of 20% human serum in a modified Connaught medium 858 (Healy, Fisher & Parker, 1955) and a growth-promoting medium (GPM) incorporating 5% of chick embryo extract in the maintenance medium. The insulin concentration used in all tests was (with one exception) 0·1 unit/ml.

In GPM the fibroblasts increased their number four to fivefold in 3 days, and their growth rate was uninfluenced by the presence of insulin. (Shih-man Chang (1955) found that cultures of human conjunctival cells showed a similar lack of response to insulin in growth-promoting medium.) However, insulin did act upon the fibroblasts by raising the ribonucleic acid content per cell and causing a lower lactic acid (LA) production per unit of glucose (G) utilized (i.e. a lower LA/G ratio).

Although the cell number of the fibroblasts increased by only 14% when kept in MM for 3 days, insulin produced a rise of 32% under the same conditions. From concurrent changes in cell and medium composition, it is clear that the response to insulin is greater when embryo extract is omitted from the medium, as reported by Chen (1954).

The HeLa cells were previously shown to respond to insulin by increasing their cell number and glucose uptake in growth-promoting medium (Leslie, Fulton & Sinclair, 1955). It is now found that the normal deoxyribonucleic acid content of

1·36 pg.*/nucleus is unaltered by the action of insulin (1 unit/ml.). When the HeLa cells were kept on MM, the effect of insulin was to increase the cell material rather than cell number. Glucose utilization and lactic acid production were greatly stimulated, but with this cell strain they kept in step and the LA/G ratio remained at its control value.

Both cell types respond to insulin by increased synthetic activity, but the HeLa cells obtained their energy solely by increased utilization of glucose, while the skin fibroblasts apparently oxidized a higher proportion of the glucose when insulin was present.

* pg. = picogram = 1×10^{-12} g.

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The *in vitro* Demonstration of Differential Corticosteroid Production within the Ox Adrenal Gland. By P. J. Ayres, R. P. Gould, Sylvia A. Simpson and J. F. Tait. (*Middlesex Hospital Medical School, London, W.* 1)

Indirect evidence obtained from the analysis of adrenal venous blood and histological examination of adrenal glands under various conditions has indicated that aldosterone is preferentially produced by the zona glomerulosa and cortisol by the zona fasciculata (Farrel, Banks & Koletsky, 1956). We have obtained direct evidence which supports this view. However, our results indicate that this differential production, at least when expressed as μg./wet weight tissue, may not apply to corticosterone in the ox gland.

Selected slices (0·3–0·35 mm. thick) from the ox adrenal cortex which, from histological evidence, were likely to consist mainly of either zona glomerulosa or fasciculata tissue, were incubated in separate Warburg vessels for 2 hr. under oxygen at 37° in 3·5 ml. Krebs–Ringer phosphate medium (Krebs, 1933) containing 13 mM glucose, 3 mM ATP and 3 mM fumarate. Eighteen slices from each zone, of approximately 200 mg. wet weight, were incubated. After chloroform extraction, 75% of the products was pooled for each type of tissue. Aldosterone, cortisol and corticosterone were analysed by an isotope dilution method, which included the extensive purification of the acetylated and/or free compounds (Ayres, Simpson & Tait, 1956*a*, *b*). The following mean values, with their estimated experimental errors, were obtained expressed as μg. steroid per g. wet tissue for zona glomerulosa and fasciculata tissue respectively: cortisol, $6{\cdot}5 \pm 0{\cdot}6$ and $16{\cdot}5 \pm 1{\cdot}5$; aldosterone, $4{\cdot}3 \pm 0{\cdot}6$ and $0{\cdot}7_2 \pm 0{\cdot}1$; corticosterone, $6{\cdot}1 \pm 0{\cdot}6$ and $4{\cdot}3 \pm 0{\cdot}4$. Aldosterone was further estimated and identified by various microtechniques including bioassay (Simpson & Tait, 1952) of the purified free compound. The other steroids were also further characterized.

25% of the products from the individual vessels were analysed separately. Student's t test showed the mean value for cortisol to be significantly higher and that for aldosterone lower ($P < 0{\cdot}001$) from the zona fasciculata compared with the zona glomerulosa. No individual estimations of corticosterone were made.

Some incubated crude capsule strippings of the gland gave aldosterone/cortisol ratios greater than 10. Histological examination showed that these usually consisted of capsule plus adhering zona glomerulosa tissue, but minimal zona fasciculata contamination.

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The Estimation of β-*N*-Acetylglucosaminidase. By Doreen Pugh, D. H. Leaback and P. G. Walker. (*Department of Biochemistry, Institute of Orthopaedics, Stanmore, Middlesex*)

The occurrence of β-*N*-acetylglucosaminidase in rat tissues has been demonstrated by a modification of the method of East, Madinaveitia & Todd (1941) in which liberation of phenol by the enzymic hydrolysis of phenyl *N*-acetyl-β-D-glucosaminide is used as a measure of the enzyme activity.

A stable, reproducible, partially purified enzyme preparation was obtained by fractionation of extracts of rat kidney homogenate buffered with citrate (0·1M, pH 4·6). The precipitate obtained between 25 and 50% saturation with ammonium sulphate, after resuspension and dialysis, contained

about 80% of the total enzyme activity of the tissue and this preparation was used to study the properties of the enzyme. The pH optimum in 0·05M citrate buffer was 4·3. The Michaelis constant (K_m) for phenyl *N*-acetyl-β-D-glucosaminide was 0·002M. With increasing substrate concentration the rate of hydrolysis increased until the reaction mixture was saturated with substrate (approx. 0·03M). The amount of hydrolysis was proportional to the time of incubation and to the enzyme concentration. The amounts of phenol and *N*-acetylglucosamine liberated by the enzyme were equivalent.

In acetate buffer (used by East *et al.* 1941) activity was much lower than in citrate and inhibition produced by acetate was shown to be competitive (K_i acetate 0·018M). The hydrolysis of phenyl *N*-acetyl-β-D-glucosaminide was also inhibited by *N*-acetylglucosamine (Watanabe, 1936), acetamide and the competing substrate ethyl *N*-acetyl-β-D-glucosaminide.

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Fatty Acid Metabolism in *Sarcina lutea*. By D. Murray and E. A. Dawes. (*Department of Biochemistry, University of Glasgow*)

The metabolism of fatty acids by the aerobe *Sarcina lutea* has been investigated. The remarkably high endogenous respiration of freshly harvested cells was reduced by vigorous aeration for 4·5 hr. (Holms & Dawes, 1955). Substrates investigated included acetate, butyrate, valerate, caproate, heptoate, caprylate, pelargonate and caprate. All were oxidized: the total oxygen uptake, less the endogenous value, corresponded to *ca.* 0·5–0·75 oxidation of added substrate, and oxidative assimilation was indicated. Fatty acid oxidation by freeze-dried cells was also studied and no appreciable difference in the rate of oxidation by these preparations was found; freeze-dried cells were used routinely thereafter.

Protoplasts of the organism have been prepared by lysozyme treatment (Weibull, 1953*a*). Neither the rate nor the total oxygen uptake of such preparations differed from those of freeze-dried cells, with caprylate as substrate. However, cell-free preparations obtained by the action of lysozyme in hypotonic media gave a very small oxygen uptake in the presence of fatty acids. Cell-free extracts were also prepared by the method of Lamanna & Mallette (1954), but no oxygen consumption was demonstrable with fatty acids using either the whole disintegrate or the supernatant from centrifugation.

Identification of the individual enzymes involved in fatty acid degradation is under investigation. Aceto-coenzyme A (CoA) kinase was present in the supernatant of Lamanna & Mallette (1954) extracts and its dependence on CoA and adenosine triphosphate (ATP) was established. Butyrate, valerate, caproate, heptoate and caprylate also reacted. This enzyme appears to be absent from the supernatant of lysed preparations. Butyryl-CoA dehydrogenase was demonstrated in the former extracts, and since the substrate used was butyrate itself, the presence of CoA and ATP was mandatory. Of the acids tested to date, there was slight evidence for acyl-CoA dehydrogenase activity with caprylate, caprate and laurate.

Studies with preparations obtained by lysozyme treatment of *S. lutea* have shown the cytochrome pigments to be associated with particulate material but 'ghosts', as described for *Bacillus megaterium* by Weibull (1953*b*), have not yet been identified.

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Fluorescent Metabolites of 3:4-Benzpyrene. By J. G. Chalmers. (*Cancer Research Department, Royal Beatson Memorial Hospital, Glasgow*)

It has been demonstrated that benzpyrene added *in vitro* is associated with the lipoprotein fraction of animal serum, while after intravenous injection it is associated in metabolized form with the albumin fraction (Chalmers, 1955). An examination of the fluorescent metabolite in the serum presents some difficulty owing to the small amount of the dose present at any one time, but bile provides a better source of fluorescent material. Metabolized benzpyrene is eliminated chiefly via the bile and faeces (Peacock, 1936; Chalmers & Peacock, 1936). Tracer experiments, however, have shown that

17% of the metabolite passes into the urine (Heidelberger & Weiss, 1951), either directly or after reabsorption from the gut.

In the present experiments benzpyrene was administered by intravenous injection, intraperitoneal injection, or by feeding in the diet. Fluorescent bile was fractionated by paper chromatography using phenol as developer and showed three zones giving fluorescent spectra maxima at (4200, 4500, 4800), (4080, 4300, 4500), and (4150, 4400, 4700) Å respectively. The zones were eluted and rechromatographed using the same solvent system without any essential change in their behaviour apart from some fading of the fastest moving zone. Mouse, rabbit, guinea pig and fowl bile all gave similar fluorescence patterns. Fluorescent material separated from the faeces of benzpyrene-treated animals, and identified as 8- and 10-monohydroxybenzpyrene by Berenblum, Crowfoot, Holiday & Schoental (1943) and by Berenblum & Schoental (1946), and unchanged benzpyrene, were chromatographed in the same solvent system but behaved differently, all moving with the solvent front.

The fluorescent material in rat and rabbit urine and serum was fractionated and gave three similar fluorescent zones. In the case of rabbit urine the eluates of all the chromatographed zones gave a positive test for glucuronic acid. Also, the absorption spectrum of the zone of highest R_F value corresponded with that of 10-hydroxybenzpyrene.

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Investigations of the Chromatographic Separation of Mixtures of 4-Dimethylamino-3:5-Dinitrophenyl (DDP) Derivatives of Amino Acids on Ion-Exchange Columns. By A. Drèze* and W. S. Reith. (*Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.* 12)

Mixtures of amino acids and peptides contained in growing cells of plant roots were separated as their DDP-derivatives on cation exchange columns (Edmunds & Reith, 1954). We now find that the resolving power of the method can be considerably increased, and also that the fundamental mechanism of the ion-exchange procedure differs from that observed generally.

In a number of experiments with cation and anion exchange resins the best results were obtained with Zeo-Karb 225. The degree of cross-linking was very important. While an 8% cross-linked resin did not retain any of the derivatives used, a 4% cross-linked resin retained them all and a good chromatographic separation on this resin can be carried out; but sharper zones were obtained with a 2% cross-linked resin. A resin that was only 1% cross-linked had lost completely the power to retain any of the derivatives which were investigated.

While generally in the ion-exchange chromatography of substances described in the literature the speed of elution increases with the molar strength of the buffer, in the case of all the DDP-derivatives examined, the speed of elution *decreased* when the molar strength of the buffer increased. Monovalent cations (Na^+, Li^+) had a selective effect on the R-values of the various derivatives, while changes in the molarity of Mg^{2+} affected the R-values of all derivatives to the same extent. But also in the case of Mg^{2+} the R-values increased with the decrease in the molarity of the buffer.

There was a fair amount of adsorption on the resin; this was overcome by the addition of organic solvents to the buffer. They also influenced selectively the R-values of the various derivatives; ethanol showed the best results so far.

There was also a marked temperature effect, the R-values increasing with the increase in temperature.

The optimal conditions found so far are: 2% cross-linked resin (grain 50–100 μ.) in Na^+-form; Na acetate buffer, pH 8, molarity 1·5, containing 20% ethanol. The exchange potentials were: aspartic acid > glutamic acid > glycine > serine > alanine > threonine > valine > leucine > tyrosine > phenylalanine > basic amino acids.

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A Method of Separation of Mixtures of DDP-Ureido Derivatives of Amino Acids and of DDP-Amino Acid Hydantoins by Paper Chromatography. By A. Drèze* and W. S. Reith. (*Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W. 12*)

Several solvent systems can be used for the separation of mixtures of DDP-ureido amino acids by paper chromatography. However, it is necessary to use phthalate-impregnated paper so as to eliminate a marked adsorption of the derivatives, and a tailing 'downwards' of the spots. The paper strips, Whatman no. 20, were dipped in a 0·05M phthalate buffer, pH 6·0, and were dried in a current of air at room temperature.

Each solvent contained one component in which the derivatives are insoluble, and one in which they are fairly soluble.

The solvent systems for the chromatography of DDP-ureido amino acids which we found to be most useful were:

No. 1. Xylene, acetic acid, phthalate buffer (0·05M, pH 6·0); 3:2:1 by volume.

No. 2. *sec.*-Butanol, phthalate buffer; 7:1 (v/v) (used with Whatman no. 1 paper).

No. 3. Toluene, chloroethanol, pyridine, phthalate buffer; 10:6:3:6 by volume.

For the chromatography of DDP-amino acid hydantoins the most useful solvent systems were found to be nos. 1 and 2, and:

No. 4. Ligroin (b.p. > 120°), pyridine; 7:3 (v/v) (on phthalate-impregnated Whatman no. 20 paper).

For all solvents, a complete equilibration of the jars was found to be most important; with solvent no. 3 it was extremely difficult to obtain reproducible R_F values, and it was necessary to run markers simultaneously under identical conditions. The other solvents gave easily reproducible R_F values.

The R_F values were found to be independent of the amount of material used, at least between 0·01 and 1·5 μmol.

As the spots are deeply coloured, a small amount of material could easily be detected.

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DEMONSTRATIONS

Apparatus for Aeration and Sampling During Growth and Metabolic Studies with Bacteria. By E. A. Dawes and W. H. Holms. (*Department of Biochemistry, University of Glasgow*)

The need frequently arises for the sampling of bacterial cultures during aerated growth, or of non-proliferating bacterial suspensions during metabolic studies under aerated conditions. Two different types of apparatus have been designed to meet such requirements (Dawes & Holms, 1956).

Type 1, for bacteria which do not sediment during the period of experimentation. A 1 l. round-bottom flask with B40 neck carries a special glass head with inlet and outlet arms. The inlet tube, which extends almost to the bottom of the flask, has a sintered glass disk distributor of 20 mm. diameter and porosity no. 1. The head is held in position by spring clips. The flask is fitted with a wide-bore drain stopcock at the bottom with a stem $\frac{1}{2}$ in. long which permits samples to be withdrawn as required and without interfering with the aeration arrangements. Additionally, the flask has a B19 socket set at an angle to the neck; this is used as an inoculation port or for the addition of substrates.

Type 2 was designed for use with *Sarcina lutea* which sediments and collects in the stopcock outlet when aeration is carried out in the apparatus described above. It consists of a 1 l. capacity pear-shaped dropping funnel with B24 neck. At the base is fitted a 3-way double oblique bore stopcock with one vertical outlet $\frac{1}{2}$ in. long and the other U-shaped. The latter tube is used as the inlet for the air stream which, entering from the base of the funnel, ensures thorough agitation. By turning the stopcock the air supply is momentarily cut off, a sample is withdrawn and aeration then resumed.

Alternatively, if the air stream is replaced by nitrogen, the apparatus may be used for anaerobic studies where sedimentation is to be avoided.

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Tissue Culture Methods in Biochemistry. By J. PAUL. (*H.E.R.T. Tissue Culture Laboratory, Department of Biochemistry, University of Glasgow*)

Simple techniques will be demonstrated for the massive culture of primary explants of animal tissues and for the serial propagation of pure cell strains.

Correlated Biochemical and Histochemical Studies on Tissue Culture Explants. By J. PAUL, A. J. HALE and ENID S. PEARSON. (*H.E.R.T. Tissue Culture Laboratory and Institute of Physiology, University of Glasgow*)

Cultures of 15-day chick embryonic heart and liver were studied at daily intervals for 1 week. Samples consisting of several explants were examined simultaneously by biochemical and histochemical methods for lipid, carbohydrate and DNA. The results of the two techniques are compared and shown to complement each other.

Components of the Acid-Soluble Fraction of Mammalian Tissues. By H. M. KEIR and J. N. DAVIDSON. (*Biochemistry Department, The University, Glasgow*)

When perchloric acid extracts of rabbit tissues are adsorbed on Dowex 1 and eluted for the separation of nucleotides by the method of gradient elution described by Hurlbert, Schmitz, Brumm & Potter (1954) it has been observed that the fraction associated with cytidine monophosphate (CMP) is invariably strongly ninhydrin-positive. When this fraction is submitted to ionophoresis on paper by the method of Davidson & Smellie (1952) the ninhydrin-positive material moves well ahead of the position normally occupied by CMP. The ninhydrin-positive material on hydrolysis yields the amino acids associated with glutathione (glutamic acid, cystine and glycine) together with an ultraviolet-absorbing base.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **351st Meeting** *of the Biochemical Society was held at the Rothamsted Experimental Station, Harpenden, Hertfordshire, on Friday, 25 May* 1956, *at* 10.45 *a.m., when the following papers were read:*

COMMUNICATIONS

Pyridoxal Phosphate as Coenzyme of Diamine Oxidase (Histaminase). By A. N. Davison. (*Roche Products Ltd., Welwyn Garden City*)

It has been found that the rate of inhibition of an enzyme by *iso*nicotinyl hydrazide under fixed conditions is a useful constant for identifying pyridoxal phosphate as a prosthetic group for a number of enzymes, particularly where the coenzyme is strongly bound to the apoenzyme (Davison, 1956).

Evidence presented by Sinclair (1952) suggested that pyridoxal phosphate was a coenzyme of histaminase. It therefore seemed interesting to investigate this possibility by the above method. Using cadaverine and histamine as substrates it has been found that the rates of inhibition by *iso*-nicotinyl hydrazide of pig's kidney diamine oxidase are similar to that of cysteine sulphinic acid decarboxylase, an established pyridoxal phosphate enzyme. Attempts to separate a coenzyme from diamine oxidase and to reactivate the dialysed enzyme with pyridoxal phosphate failed. However, it proved possible to reactivate diamine oxidase partially with pyridoxal phosphate after the enzyme had been inhibited by *iso*nicotinyl hydrazide and dialysed.

Additional evidence was also obtained indicating that diamine oxidase and histaminase in this preparation are the same enzyme (see also Zeller, 1951). It thus seems probable that pyridoxal phosphate is coenzyme to diamine oxidase, an enzyme oxidizing both short-chain diamines and substrates such as histamine.

I am grateful to the directors of Roche Products Limited for permission to publish this work.

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The Production of Coproporphyrin Precursors by a *Rhodopseudomonas* sp. By R. Cooper (introduced by D. D. Woods). (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Free porphyrins, mainly coproporphyrin III, are excreted by suspensions of *Rhodopseudomonas spheroides* incubated in the light with glycine and α-oxoglutarate or succinate; addition of iron salts prevents the production of porphyrin but increases the formation of bacteriochlorophyll (Lascelles, 1956).

A related photosynthetic bacterium (*Rhodopseudomonas*, species not identified) has been isolated from a local pond. Suspensions of this organism incubated anaerobically in the light with glycine and succinate formed very little free porphyrin, but a colourless precursor was produced. In the presence of air the amount of precursor was reduced and considerable amounts of porphyrin appeared. Bacteriochlorophyll was formed both aerobically and anaerobically. The supernatant fluids derived from anaerobic incubations turned yellow and then red on standing in air; there was a simultaneous 30-fold increase in porphyrin content (mainly coproporphyrin III) as measured by the optical density at 403 mμ. in 1·4N-HCl. At the same time an absorption band at 500 mμ. appeared, and the optical density at this wavelength rapidly increased and then decreased as the optical density at 403 mμ. was rising. This sequence of changes was accelerated by acid, alkali, hydrogen peroxide, iodine, ferric chloride or light (especially ultraviolet), and retarded by ascorbic acid or cysteine. The supernatants were Ehrlich-negative.

A preparation of the substance absorbing at 500 mμ. was made by rapid ether extraction from 0·2N-HCl solution and subsequent precipitation from an aqueous solution at pH 3·5–4·0. A solution in 1·4N-HCl had a strong, broad absorption peak with a maximum at 500 mμ. in addition to the absorption curve of residual porphyrin. On standing the solution in air, the optical density at 500 mμ. decreased whilst that at 403 mμ. increased.

Cell-free extracts of both this organism and *Rh. spheroides* produced similar coproporphyrin precursors when incubated anaerobically with δ-aminolaevulic acid.

The colourless precursor and that absorbing at 500 mμ. may be intermediates in the conversion of δ-aminolaevulic acid to coproporphyrin.

Bogorad (1955) has recently reported that spinach leaf preparations convert porphobilinogen to a colourless product which is further converted to uroporphyrin I through an intermediate with a strong absorption band at 500 mμ.

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Cobalamin and the Synthesis of Methionine by Cell-free Extracts of *Escherichia coli*. By C. W. Helleiner* and D. D. Woods (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Gibson & Woods (1952) have shown that suspensions of a mutant strain of *Escherichia coli* deficient in cobalamin synthesize methionine from homocysteine only if cobalamin is added. Cross & Woods (1954) demonstrated the synthesis of methionine from homocysteine and serine by cell-free extracts of another mutant of *Esch. coli*, which required serine or glycine for growth. Extracts of this type were further investigated by Szulmajster & Woods (1955), but no increase in methionine synthesis occurred on the addition of cobalamin.

Cell-free extracts of a mutant of *Esch. coli* requiring either methionine or cobalamin for growth, no. 121/176 of Davis & Mingioli (1950), have now been prepared. The organisms were harvested from a medium containing a suboptimal amount of methionine, and suspensions (about 100 mg. dry wt./ml.) subjected to ultra-sonic treatment. The supernatant fluid after centrifuging was dialysed. Such extracts were more active than those obtained from acetone-dried cells.

When this preparation was incubated for 3 hr. with 10^{-2} M-DL-homocysteine, 10^{-2} M-DL-serine, 5×10^{-3} M-$MgCl_2$, 2×10^{-3} M adenosinetriphosphate, 10^{-1} M phosphate buffer (pH 7·3) and a heated extract of *Esch. coli*, from 4 to 5 μmmoles of L-methionine/mg. protein (of the enzymic preparation) was synthesized. This was increased to 15–18 μmmoles/mg. protein by adding cyanocobalamin (10 mμg./ml.). In the presence of cyanocobalamin, the omission in turn of homocysteine, serine, heated extract of *Esch. coli* and adenosinetriphosphate plus Mg^{2+} reduced methionine synthesis to about 2, 3, 4 and 8 μmmoles/mg. protein respectively.

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* Post-doctoral Fellow, National Research Council of Canada.

The Oxidation of Glucose by *Acetobacter suboxydans* By J. A. Fewster* (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

Aerobic growth of *Acetobacter suboxydans* (A.T.C.C. 621) on media containing glucose and chalk (in suspension) results in the accumulation of the calcium salts of 2-oxo- and 5-oxogluconic acid in the proportion of 1:10 (see also Kulka & Walker, 1954). Washed organisms grown for 72 hr. in this manner oxidize glucose at pH 6·0 and 30° to a stage well beyond that of the oxo acids; manometric measurement shows an O_2 uptake of 3·5 moles and a CO_2 output of 4 moles/mole of substrate. Gluconic, 5-oxo- and 2-oxogluconic acid are formed, and the first two acids are oxidized in a manner compatible with their being true intermediates. The corresponding phosphorylated derivatives have not been detected and compounds representing further stages of oxidation have not been identified. 3-*O*-Methyl-D-glucose, D-glucosone, D-galactose, D-xylose and L-arabinose consume 0·5 and D-mannose 1 mole of O_2/mole; D-glucurone and glucose 6-phosphate are not attacked.

The oxidation of glucose is little inhibited by 10^{-3} M arsenite or 10^{-4} M dinitrophenol, but a concentration of 10^{-3} M dinitrophenol stops O_2 uptake at a value corresponding to the formation of the oxo acids. Appreciable inhibition results with 10^{-3} M cyanide and 5×10^{-2} M fluoride.

In agreement with Butlin (1936) it has been shown that when *A. suboxydans* is grown for 72 hr.

* Guinness Research Fellow in Microbiological Biochemistry.

on a medium containing glucose in the absence of a neutralizing agent the washed organisms possess a very limited oxidizing ability; they oxidize only glucose, with the quantitative production of gluconic acid. Full activity is not restored by addition of DPN, TPN, CoA, or a hot-water extract of organisms harvested from a medium containing chalk. In contrast to the active organisms, which are pink and rich in a single cytochrome component described by Smith (1954*a*, *b*) as being characteristic of *A. suboxydans* (α-band 554 mμ., β-band 527 mμ.), the organisms grown in the absence of chalk are cream coloured and contain, as the only cytochrome-like constituent, a small amount of material exhibiting absorption bands at 559 and 530 mμ., measured after immersion in liquid air. The presence of such a cytochrome in *A. suboxydans* has not previously been reported.

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Incorporation of ^{35}S-Sulphate by Guinea Pig Colon *in vitro*. By C. A. Pasternak, P. W. Kent and R. E. Davies. (*Department of Biochemistry, University of Oxford*)

The gastro-intestinal tract is known to be an active site of ^{35}S-sulphate fixation. Experiments on animals with intestinal fistulae given ^{35}S-sulphate intravenously have shown that the secretion of the duodenum contains a sulphated mucopolysaccharide having a high rate of turnover (Kent, Whitehouse, Jennings & Florey, 1956). The present work describes an *in vitro* technique for the study of ^{35}S-sulphate incorporated by guinea pig colon.

Lengths of colon were incubated in oxygenated Krebs–Ringer buffer containing $Na_2{}^{35}SO_4$. After 3 hr. the tissue was removed from the surrounding medium and extracted with 2% NaOH. Medium and extract were then dialysed against running tap water to remove free inorganic sulphate. Carrier Na_2SO_4 was added until constant activity was attained. Both medium and tissue extract were radioactive at the end of dialysis; this indicates the extrusion of a non-dialysable ^{35}S-labelled component by the tissue during incubation. Approximately half the total activity of non-dialysable material was in this soluble form. Gastric mucosae did not incorporate ^{35}S-sulphate under similar conditions.

Addition of 0·02M glucose to the incubation medium raised the Q_{O_2} slightly and increased the labelling of both extrudate and tissue extract several-fold (cf. Boström & Jorpes, 1954). Glucosamine, galactosamine, *N*-acetylglucosamine, potassium methane sulphonate and potassium ethyl sulphate (all 0·02M) were without effect on oxygen uptake or sulphate incorporation. Sodium fluoroacetate, phenol and salicylic acid (0·02M) abolished respiration almost completely; their action on sulphate uptake was similar. Lower concentrations of salicylic acid (0·0005–0·005M) were without effect (cf. Boström & Månsson, 1953; Kodicek & Loewi, 1955). Anaerobic incubation, and pretreatment of the tissue by heating or freezing, prevented any incorporation of ^{35}S-sulphate.

Extruded mucin in the absence of tissue did not fix ^{35}S-sulphate, which suggests that the incorporation processes are intracellular.

The ratio of sulphate incorporated/total non-dialysable sulphate was found to be higher for the extruded fraction than for the tissue extract, though in no case did it exceed 5%. Such an increase would not be detected by the analytical methods employed for the estimation of sulphate or hexosamine; it is therefore impossible to say whether the incorporation of ^{35}S-sulphate represents a synthesis of new material or a ^{35}S-sulphate exchange at pre-existing sites.

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Oxidation of Octanoic Acid by Cell-free Bacterial Extracts. By S. Dagley. (*Department of Biochemistry, University of Leeds*)

Studies with extracts from the anaerobic organism *Clostridium kluyveri* (Stadtman & Stadtman, 1953) have shown that certain fatty acids are oxidized to acetate by processes similar in essentials to those in mammalian systems, but the mechanisms employed by aerobic bacteria have remained obscure. Thus, although results with whole cells of *Serratia marcescens* (Silliker & Rittenberg, 1952) did not apparently accord with β-oxidation, it has been shown more recently that extracts from *Pseudomonas fluorescens* (Ivler, Wolfe & Rittenberg, 1955) catalyse the formation of acetate from decanoate

with the uptake of 1 μmole of O_2 per μmole of substrate. In experiments with cell-free extracts prepared from a vibrio (Happold & Key, 1932) we have now been able to show that octanoic acid is oxidized by molecular O_2 to two-carbon units; that diphosphopyridine nucleotide, coenzyme A and Mg^{2+} ions are cofactors in this process; and that subsequent oxidation most probably follows the tricarboxylic acid cycle. After growth with forced aeration in a mineral salt medium supplying octanoic acid as sole source of carbon, each 10 g. (wet weight) of cells were crushed in a Hughes press at $-14°$ and whilst still frozen they were ground in a mortar to a smooth paste with 10 g. of alumina and 25 ml. of phosphate buffer. Centrifugation to remove alumina and cell debris gave clear extracts which contained about 18 mg. protein/ml. and catalysed the oxidation of octanoate. The rate of oxidation decreased with time and the volume of O_2 utilized approached that for complete oxidation, namely, 11 moles O_2 per mole octanoate, when fumarate was added: thus 4 moles O_2 had been utilized in excess of the control after 40 min. and 8 moles O_2 after 150 min.

Uptake of O_2 by these extracts in the absence of added substrate was high, but was largely abolished by dialysis. Additions of Mg^{2+} and DPN were then necessary for activity and only 2·5–3 moles O_2 were consumed per mole octanoate. Further, volatile acid was produced in the reaction and identified as acetic acid by vapour-phase chromatography (James & Martin, 1952). Both O_2 uptake and acetate production fell somewhat short of the values required by the equation

$$C_8H_{16}O_2 + 3O_2 \rightarrow 4CH_3COOH,$$

but the acetic acid that accumulates during the reaction is inhibitory to dialysed extracts. No keto acid was detected. 'Dowex-1' treatment gave extracts whose activity was increased by addition of coenzyme A.

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Conditions for Zone Electrophoresis of Parathyroid Hormone. By A. H. Gordon. (*National Institute for Medical Research, London, N.W.* 7)

Since the successful separation of mixtures of various proteins by means of electrophoresis on columns of powdered cellulose (Porath, 1954; Campbell & Stone, 1956) it seemed possible that this method might be of value for purification of the parathyroid hormone. Although by Tiselius's electrophoresis it had previously been possible to demonstrate (L'Heureux, Tepperman & Wilhelmi, 1947) the inhomogeneity of the best preparations then available, successful purification by zone electrophoresis has not yet been achieved. Meanwhile, conditions for ultrafiltration of the hormone have been found (Davies & Gordon, 1955), and thus material of considerably lower molecular weight than that used by L'Heureux *et al.* is now available for electrophoresis.

Although powdered cellulose is often regarded as an inert support, noticeable amounts of protein can readily be shown to remain adsorbed on it even after thorough washing with buffer. Because it was feared that behaviour of this kind might be even more marked with parathyroid hormone preparations than it is with the serum proteins, an experiment to measure adsorption was carried out. For this trial 19 mg. of ultrafiltered material was applied to a column 40 × 2 cm. Recovery after elution with 0·1 M-NaCl, 0·05 N acetic acid was only 29%. However, when a further 7·4 mg. of the same material was applied, recovery rose to 78%, and since even higher recoveries seemed to be likely on further use of the same column, it was decided to attempt an electrophoresis. For this purpose 26 mg. of the same material dissolved now in 0·05 M-NaCl, 0·05 N acetic acid was used. Electrophoresis, which took place in the direction of the cathode, was carried out for 18 hr. with a current of 22 mA. Examination of the eluate proteins in the ultraviolet showed two partially resolved peaks with $E^{250}/E^{275} = 0·5$. In addition, a small amount of material with $E^{250}/E^{275} \simeq 0·9$ was found to have emerged both in front of and behind the main bands. Biological assay by the mouse urine phosphate method (Davies, Gordon & Mussett, 1955) showed that the urine phosphate activity was associated with the two main bands (tested together) and not with the material having $E^{250}/E^{275} \simeq 0·9$.

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The Sulphation of Phenol by Granulation Tissue. By G. Loewi and P. W. Kent. (*Department of Pathology, Radcliffe Infirmary, Oxford and Department of Biochemistry, University of Oxford*)

Kodicek & Loewi (1955) have reported the formation *in vitro* of a sulphated mucosubstance by granulation tissue. It was therefore of interest to determine whether this tissue was also capable of synthesizing other *O*-sulphates. The conjugation of phenol as the sulphate and as the glucuronide was demonstrated by De Meio & Arnolt (1944) in liver. No such conjugates appear to be formed by spleen, heart, kidney or hepatoma (Segal, 1955). Dodgson & Spencer (1954) have described a chondrosulphatase and an aryl sulphatase in the periwinkle (*Littorina littorea*).

Granulation tissue (Kodicek & Loewi, 1955) was sliced free-hand and incubated at 37° in Krebs–Ringer buffer (pH 7·4) containing 4 μc $Na_2{}^{35}SO_4$, 0·1 % (w/v) glucose and 10^{-3} M phenol (final concentration) in pure oxygen. After shaking for 3 hr., the tissue was removed, dried and weighed. The residual medium, after extraction several times with ether to remove free phenol, was concentrated to dryness under reduced pressure. The residue was extracted with ethanol and the resulting extract was examined chromatographically on Whatman no. 1 paper using *n*-butanol–ethanol–water (4:1:5, v/v).

After treatment with HCl gas and heating at 100° the papers were sprayed with a solution of diazotized sulphanilic acid and exposed to ammonia vapour. The media extracts showed a single component as an orange-yellow spot, R_F 0·47 (identical with authentic phenol sulphate) and having, in different experiments, a radioactivity of between 200 and 300 counts/min. The same compound was obtained from liver slices but with approximately ten times greater radioactivity. When non-radioactive phenol sulphate was added to the ethanolic extracts, one coincident coloured and radioactive component (R_F 0·47) was found. Inorganic sulphate ions were immobile in this system.

No phenol sulphate was detected when phenol was omitted from the incubation medium nor when boiled tissue was employed. The presence of phenol did not diminish the Q_{O_2} of granulation tissue.

It is concluded that granulation tissue is capable of sulphating not only carbohydrate substrates but also phenol. Further work is necessary to show the nature and relationship of the enzymes involved.

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Biochemical Genetics of the Flower Pigments of *Primula sinensis*. By Thelma O. Dayton, J. B. Harborne and H. S. A. Sherratt. (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

The biochemical genetics of the flower pigments of *Primula sinensis* was earlier investigated at the John Innes Horticultural Institution (Scott-Moncrieff, 1936). Red flowers (*K*) contained malvidin 3-galactoside which was dominant to pelargonidin 3-glycoside in orange flowers. A factor *Dz* controlled the independent formation of pelargonidin in the presence of *K*. Other genes influenced flower colour by the production of a flavone co-pigment, and by altering the pH of the cell sap and the concentration of anthocyanin.

A reinvestigation of different genotypes of *P. sinensis* by modern methods has revealed a more complex situation. In general, *K* types contain malvidin, delphinidin and petunidin; these may be present as more than one glycoside. Two glycosides of kaempferol are present, only one of which appears to act as a co-pigment. An unidentified flavanone glycoside has also been isolated in crystalline form. The names sinensetin and sinensin are provisionally proposed for the aglycone and glycoside respectively. Sinensetin from its absorption spectra in neutral and alkaline solutions and its colour reactions, does not appear to be related in its *A*-ring hydroxylation pattern to any of the other phenolic pigments present, even though it apparently competes with these other pigments for precursors. This is in contrast to the situation in *Antirrhinum majus* in which plant all the pigments present are closely related in structure and in gene control (Geissman, Jorgensen & Johnson, 1954; Dayton, 1956).

The genetical results (Dayton, 1954) show some new interactions between the various genes controlling pigment production which can be interpreted in terms of competition for precursors.

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Biochemical Genetics of the Acylated Anthocyanins of Tuberous Solanums. By J. B. HARBORNE (introduced by H. S. A. SHERRATT). (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

As part of an investigation of the inheritance of colour in potatoes, Dodds & Long (1955) found acylated glycosides of pelargonidin, paeonidin and petunidin to be the main pigments present in cultivated diploid species. The first two anthocyanins were present in red tubers (*ppRR*) and the last two in blue tubers (*PpRR*). The presence of a gene for acylation, which behaved as a simple Mendelian factor, was later observed by the same authors (unpublished work). Plants with non-acylated pigments had either deep red or deep blue tubers and flowers according to their genotypes.

The pigments of the red, deep red, blue and deep blue tubers have now been studied in detail, using chromatographic and spectrophotometric methods, as described for similar investigations of phenolic pigments (Geissman, Harborne & Seikel, 1956). Acid and alkaline hydrolyses of the carefully purified eluates of the separated pigments gave information regarding glycosidation and acylation respectively. The results obtained indicate that the acylating gene has at least four biochemical effects on pigment production. Besides controlling acylation of the anthocyanins with *p*-coumaric acid, it changes the positions of the sugars (arabinose and glucose) from 3-pentose glycoside in the recessive state to 3:5-dimonoside. Also in its recessive state, the production of paeonidin is suppressed and instead cyanidin is formed in deep red tubers, delphinidin in deep blue tubers.

Measurements of the absorption spectra of the purified anthocyanins in the range 250–600 mμ. indicate not only the presence or absence of the acyl group but also that the ratio of *p*-coumaric acid to anthocyanin is 1:1. Other results suggest that the acyl component is linked to the anthocyanin through a sugar residue.

Cyanidin in a non-acylated form, probably as the 3-rhamnoglucoside, and rutin (quercetin 3-rhamnoglucoside) occur chiefly as flower pigments in plants of all four of the above genotypes. This suggests that in potatoes the biosynthetic pathway to cyanidin diverges at an early stage from the pathway to the other anthocyanins present.

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Genetics of Pathogenicity in *Fusarium oxysporum*. By E. W. BUXTON (introduced by R. H. KENTEN). (*Rothamsted Experimental Station, Harpenden, Herts*)

Irradiating conidia of *Fusarium oxysporum* f. *pisi*, the cause of pea wilt (Linford, 1928; Snyder, 1935; Buxton, 1955), with ultraviolet light alters their nutritional requirements *in vitro*, and these can be classified into specific needs for amino acids, yeast nucleic acids or vitamins. Fifty-one mutants, derived from analysis of 2119 irradiation survivors, were used as genetic markers for synthesizing heterokaryons (colonies with different nuclear types associated in the same hyphae) between differently virulent strains of the fungus.

Avirulent mutants formed heterokaryons, by germ tube anastomosis (Buxton, 1954), which had virulence restored to the same level as that of the original pathogenic wild type isolates. When heterokaryons were made between nutritional mutants derived from pathogenic races 1 and 2 (distinguished by their abilities to wilt different pea varieties), three prototrophic conidia occurred in 10^8 conidia from a heterokaryon. These three conidia were easily recognizable because they grew like wild types on non-supplemented medium. Progeny from the prototrophic colonies contained a small proportion of segregants and recombinants which were detected by their nutritional requirements, their colour on a non-nitrogenous modified Czapek-Dox agar medium, and their differential tolerance towards an actinomycete inhibitor (Buxton & Richards, 1955). When recombinants were tested for pathogenicity on pea varieties known to differentiate the three physiologic races of the fungus, some wilted a variety resistant to races 1 and 2, thus resembling the wild type race 3. This suggests a possible mode of origin of physiologic races in *Fusarium*. The underlying genetic mechanism, which resembles the para-sexual cycle described in *Aspergillus* and *Penicillium* spp. (Pontecorvo, Roper & Forbes, 1953; Pontecorvo & Sermonti, 1954), together with the effect of heterokaryosis on virulence, might explain much of the variability prevalent in the pathogenicity of this and certain other Fungi Imperfecti.

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The Effect of Nitrogen Mustards on the Incorporation of Labelled Amino Acids into Protein by *Staphylococcus aureus*. By A. R. Crathorn and G. D. Hunter. (*The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W. 3*)

The modes of action of radiomimetic agents employed in cancer therapy are not well understood. In the course of a search for a system where direct biochemical effects could be demonstrated, the action of nitrogen mustards on the utilization of amino acids by *Staphylococcus aureus* Duncan has been investigated.

Gale & Folkes (1953) have shown that in addition to the normal assimilation of amino acids that takes place under conditions of net protein synthesis (condition 1), a further type of amino acid incorporation into protein can occur in this organism. In the absence of a complete source of essential nitrogen compounds, individual amino acids are incorporated into protein by *Staph. aureus* by what appears to be an 'exchange reaction' with amino acid residues in the protein molecules already present (condition 2). Our results show a marked correlation between the inhibition of amino acid incorporation under condition 2 and the activity of the nitrogen mustards in causing tumour inhibition. Under the same conditions and time of exposure to the chemical agent, the effect of even the most active nitrogen mustards on incorporation of amino acids under condition 1 is small. Similarly, the inhibitory effects obtained are not explicable in terms of a loss of viability.

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Isolation of D-*glycero*-D-*galacto*-Heptose from the Specific Polysaccharide of *Chromobacterium violaceum* (BN). By A. P. Maclennan and D. A. L. Davies. (*Microbiological Research Establishment, Porton, Wilts*)

Only in recent years have aldoheptose sugars been found in nature (Jesiatis & Goebel, 1952; Slein & Schnell, 1953; Fromme, Lüderitz & Westphal, 1954; Weidel, Koch & Bobosch, 1954; Davies, 1955; Davies, Morgan & Record, 1955; Crumpton & Davies, 1956; Davies, 1956). In only one of these investigations (Slein & Schnell, 1953) was the sugar identified (L-*glycero*-D-*manno*-heptose).

The specific polysaccharide of *Chromobacterium violaceum* (BN) contains an aldoheptose sugar (Davies, 1955). A preparation of the somatic antigen of this organism was obtained from acetone-dried bacteria by extraction with trichloroacetic acid (Boivin, Mesrobeanu & Mesrobeanu, 1933). A degraded polysaccharide hapten obtained from the antigenic complex was shown by paper chromatography to have three components, an amino sugar, a methyl pentose and an aldoheptose.

A salt-free hydrolysate of the polysaccharide was placed on a resin column (Zeo-Karb 225) and the neutral sugars were eluted with water. The amino sugar was subsequently eluted with 0·3N-HCl, crystallized and identified as D-glucosamine. The solution of neutral sugars was placed on a column of cellulose powder; on eluting with 95% (v/v) acetone the methylpentose emerged first. This was crystallized and identified as L-rhamnose. A later fraction which contained the aldoheptose was evaporated to a syrup and a sample, after drying under acetone, showed a rotation, $[\alpha]_D^{20} = +61°$. This approached the value of D-*glycero*-D-*galacto*-heptose (+68·6°) but differed from the values recorded for other heptose sugars. In a variety of solvent systems the sugar corresponded in position on paper chromatograms with D-*glycero*-D-*galacto*-heptose and differed in position in at least one solvent system from seven other synthetic heptoses which were available for comparison. Crystallization proved difficult, but a small yield of heptose crystals was finally obtained from 90% (v/v) ethanol. The infrared absorption spectrum of the isolated sugar differed markedly from those of seven different aldoheptoses but was identical with that given by an authentic sample of D-*glycero*-D-*galacto*-heptose.

We are greatly indebted to Professor N. K. Richtmyer for generous samples of aldoheptose sugars, and to Mr L. C. Thomas for carrying out the infrared spectroscopy.

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The Biosynthesis of Ergothioneine by *Claviceps purpurea*. 2. The Incorporation of [^{35}S]Methionine and the Non-Utilization of [2-^{14}C]Histamine. By JENNIFER WILDY and H. HEATH. (*University College Hospital Medical School, London, W.C.* 1)

The incorporation of [2-^{14}C]acetate into ergothioneine by *Claviceps purpurea* has been previously reported by Heath & Wildy (1956). This showed clearly that there was a direct relationship between ergothioneine and histidine biosynthesis. We now wish to report further work on the use of other potential precursors.

[^{35}S]Ergothioneine was isolated from a 10-day shake culture of *C. purpurea* grown as previously described. The sulphur source consisted of $MgSO_4$ (0·03%) and [^{35}S]methionine (0·004%). The biosynthetic pathway was not further elucidated, however, by this incorporation of isotope since this fungus is capable of utilizing sulphate for its entire sulphur requirements and it was shown that part, at least, of the labelled methionine had been oxidized to sulphate in the medium. Thus the isotope in the ergothioneine could have been derived from sulphate or any of the other sulphur compounds which are themselves derived from sulphate, and not necessarily from the labelled methionine. The addition of inactive pools of sulphur compounds adversely effected the growth of the fungus and the identity of the immediate sulphur precursor of ergothioneine has not so far been established.

From our previous work on the incorporation of [2-^{14}C]acetate it was apparent that the synthesis of ergothioneine and histidine were closely related. As [2-^{14}C]histamine was readily available and histamine is itself a constituent of ergot, the effect of this compound on the system was studied. Both the ergothioneine and histidine isolated from a culture of *C. purpurea* grown on [2-^{14}C]histamine were unlabelled. Although histamine is not a precursor of ergothioneine this work has thrown light on the metabolism of the imidazole ring system by this fungus because all the residual activity was associated exclusively with imidazole compounds. There was no degradation of the imidazole to yield a one carbon fragment derived from the C-2 of the ring. Neither did free histamine remain in the culture broth; the majority of the activity was found in at least three diazo-reacting compounds. Paper chromatography of the broth before and after hydrolysis showed that one compound was imidazoleacetic acid but the other two compounds could not be identified. The recent publication of Karjala, Turnquest & Schayer (1956) shows that the principal urinary metabolites of histamine in the mouse, besides imidazoleacetic acid, are 1-ribosylimidazole-4-acetic acid and 1-methylimidazole-4-acetic acid.

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An Indole–Alkylamine Regulating Heart-beat in Crustacea. By D. B. CARLISLE. (*The Plymouth Laboratory of the Marine Biological Association of the United Kingdom*)

The pericardial organs of Crustacea are neurohaemal organs consisting primarily of nerve endings, with some connective tissue, and lie free in the blood. Histological evidence suggests that they secrete a neurohumour into the blood. Extracts of these organs contain a principle which regulates heart beat. There is evidence that this substance is in fact released into the bloodstream and removed from the blood by the heart. Addition of an equal amount of N-NaOH to an extract under aerobic conditions leads to a transient green fluorescence, similar to that given by catechol amines. The activity is destroyed by amineoxidase and more slowly by *ortho*-diphenol oxidase, prepared from potatoes. Of

a large range of amines tested on crustacean hearts only 5-hydroxytryptamine had a similar effect under all conditions to the extract of pericardial organs. Paper chromatography yielded two spots, one with a lower and one with a higher R_F value than 5-hydroxytryptamine, but gave no evidence for the presence of this substance. On elution both of these spots proved active. Both gave the same colour reactions as 5-hydroxytryptamine and are certainly indole-alkylamines. One is destroyed by *ortho*-diphenol oxidase and may be presumed to be an *ortho*-dihydroxyindole-alkylamine. The other is destroyed by this enzyme, but far more slowly. If a fresh extract is left standing the second substance disappears to be replaced by a roughly equivalent amount of the first substance. This may be the precursor substance, presumably with one of the hydroxyl groups blocked in some way. It seems most plausible from the biological evidence taken in conjunction with the pharmacological and biochemical evidence that the active substance is an *ortho*-dihydroxytryptamine.

DEMONSTRATIONS

The Rothamsted Farm. By H. V. Garner (introduced by R. H. Kenten). (*Field Experiments Section, Rothamsted Experimental Station, Harpenden, Herts*)

The Rothamsted Experimental Station was founded by Sir John Bennett Lawes, a Hertfordshire landowner, in 1843. He had already set himself up as a fertilizer manufacturer and taken out a patent for superphosphate—his own invention. He had the vision to foresee the importance of inorganic salts as sources of plant nutrients, and at the same time he had the agricultural knowledge which convinced him that the most practical way of studying crop nutrition and impressing his findings on his fellow farmers was to set up a system of field experiments designed to last for a period of years.

Thus began the classical experiments on wheat, barley, mangolds and grass which still survive. Rothamsted is now a major centre of field experimentation in this country, and it possesses not only the unrivalled series of old plots with their vivid contrasts due to long periods of differential manuring, but also numerous experiments based on statistical layouts.

The Rothamsted farm in early summer shows the effects of nitrogenous fertilizers on cereals and grass, as they occur on the old fields. The effects of phosphorus and potassium are less visible at this stage, though the classical barley plots generally show earlier growth where phosphate is supplied.

The layout can be seen of more complicated experiments in which the comparison lies not between fertilizers but between systems of farming. Briefly, the problem is to determine whether one kind of rotation maintains soil structure and soil nutrients more than another. Any lack of soil nutrients can easily be repaired but the loss of structure can be a much more serious matter.

Many of the experiments deal with the control of plant diseases caused by virus and fungi and are closely linked with the laboratory activities of the biological staff.

Apart from the plots, which number about 2000, there is a large range of buildings equipped with the machines and facilities necessary for experimental work, and a productive commercial farm, whose fields can be drawn on for further experiments as required.

The Large-Scale Production of Leaf Protein. By M. Byers, D. Fairclough and N. W. Pirie. (*Rothamsted Experimental Station, Harpenden, Herts*)

Our aim is to separate protein from the fibrous and strongly flavoured components of the leaf and so make, from material suitable only as food for ruminants, a food for non-ruminants including man. The by-products will also be used, but the demonstration is limited to protein. Most types of fresh, lush leaves can be used and extraction is as a rule better the greater the water and protein content of the leaf.

Pulping takes place in a drum containing beaters fixed to an axial shaft driven at 400–1500 r.p.m. according to the texture of the crop. The rate of movement through the drum is controllable so that the amount of work done can be varied with consequent variation in the extent of extraction and the quality of the protein extracted.

Pulping consumes 3–10 kwh./ton of wet leaf and efficiency depends on optimal feeding. The feeder is

therefore controlled by relays in the circuit of the pulper motor so that the feed varies and keeps power consumption fairly constant. The pulp falls on to a perforated conveyor belt and travels into an automatic hydraulic press. In this, the conveyor moves only when no pressure is on it and pressure can be maintained for as long as necessary. The first factor makes this press superior to screw expellers and the second to presses in which rollers press the material. About a third of the protein comes out in the juice and a further quantity comes out if the extraction is repeated.

Leaf proteins coagulate between 50° and 80°. Fractions with different properties can therefore be made, but for convenience we heat continuously with steam to 75–80° in an automatic unit which adjusts inflow to keep outflow temperature constant. Alternatively, protein can be coagulated by ageing, acidification or shaking with water-immiscible solvents. After heating, protein filters off easily in vertical cloth sleeves. The mass of protein is pressed to 40% DM, shredded, suspended in water and pressed again.

In this form it has to be kept under refrigeration but can be kept at room temperature if dried or extracted with acetone or methanol. After extraction it is pale green or fawn, tasteless, and contains 10–13% N, i.e. it is 60–80% protein. The remainder is starch, fat, fibre and minerals in varying amounts depending on the original crop and the care exercised in the preparation.

Electron Microscopy. By H. L. Nixon (introduced by N. W. Pirie). (*Rothamsted Experimental Station, Harpenden, Herts*)

The microscope is an RCA type B instrument with many home-made modifications; the resolving power under everyday conditions is about 30–40 Å, with a best performance of perhaps 20 Å. The Metropolitan-Vickers evaporating plant has been modified to make it suitable for making evaporated carbon replicas and support films in addition to the usual shadow-casting with platinum or palladium. There is a Cook and Perkins ultramicrotome.

1. *The use of electron microscopy for counting virus particles.* The spray droplet method of Backus & Williams (1949) has been used in few laboratories because of practical difficulties. These have been overcome by modifications to the original method, and particle counting by electron microscopy is now used, together with serological and infectivity tests, in studies of virus multiplication under different conditions.

Counting is particularly useful at low concentrations, and solutions containing down to 0·1 mg./l. or about 10 particles of tobacco mosaic virus (TMV)/ml. can be assayed without preliminary concentration. This is below the limit for serological tests. With higher concentrations there is excellent agreement of the counts with the end points of precipitin tests made with the same samples from a dilution series of purified TMV.

2. *Thin sectioning of plant tissue.* The standard techniques for ultra-thin sectioning are difficult to apply to plant material because penetration of solutions into the tissues is very slow, even from cut edges. This makes the use of very narrow strips essential, and, even so, preservation is worse than that obtained with animal tissues. In making a study of the way in which the submicroscopic structures in the cell are affected by virus infection, the aim is to find the site or sites of virus multiplication within the cell. Characteristic changes in the internal structure of some chloroplasts in tobacco leaf parenchyma cells have been observed in tissue from plants carrying a systemic infection of TMV. Progress is slow, because TMV rods do not stain with osmium from the fixative and are therefore difficult to see in sections examined with the methacrylate embedding still in place. If the embedding material is removed by solution and the preparation shadowed, the rods can be seen easily, but the process of solution destroys fine structure and makes interpretation difficult.

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Pot Culture and Solution-Culture Experiments in Glasshouses. By D. J. WATSON (introduced by N. W. PIRIE). (*Rothamsted Experimental Station, Harpenden, Herts*)

The pot culture experiments deal with the following subjects:

(1) An attempt to diagnose the nutrient status of soil by measuring nutrient uptake of excised roots transferred from the soil to standard laboratory conditions; the material used is barley grown in Rothamsted soil with varying supply of N, P and K fertilizers.

(2) Onset of dormancy in the seeds of the winter wild oat (*Avena ludoviciana*) during the ripening of the panicles.

(3) Effect of applying nitrogenous fertilizer to barley at sowing on the response to a late N application at ear emergence.

(4) An analysis of the effect of N, P and K on leaf growth of sugar beet, in terms of the rate of production, longevity and size of leaves.

(5) Effect of variations of soil moisture content between field capacity and wilting on the growth of sugar beet.

The solution-culture experiments are concerned with:

(1) The effect of excising root tips or varying fractions of the root system on subsequent growth of roots and on nutrient uptake.

(2) Influence of boron on transport of carbohydrate from leaves.

(3) Use of various iron complexes as sources of iron in culture solutions of varying pH.

(4) Interaction of molybdenum with iron supplied in different forms (chloride, citrate, Fe-EDTA).

Aphid Dispersal. By C. G. JOHNSON (introduced by N. W. PIRIE). (*Rothamsted Experimental Station, Harpenden, Herts*)

Trapping techniques. During recent years we have employed various methods in the trapping of airborne insects at different altitudes, e.g. nets on a barrage-balloon cable up to 4000 ft. and electrically operated suction traps. One of the latter is on display; it consists of a fan sampling air at a constant rate. The insects in the air are deposited in a tube; disks drop automatically into this tube, so dividing up the catch at pre-set time intervals.

Flight of aphids from the crop. Photographs show the fluctuations in numbers of aphids flying from hour to hour over a crop heavily infested with aphids. There is typically a bidiurnal periodicity of flight.

Dispersal in the upper air. Aphids become generally dispersed by turbulent diffusion to great heights in the air. The density diminishes with altitude. It has been necessary to construct extremely large and powerful traps in order to sample adequately the low concentrations of aphids found at the higher altitudes. Several traps sampling at different altitudes simultaneously (up to 1000 ft.) enable the aphid density gradient to be estimated. Diagrams show that in general the air is populated and depopulated at least once in 24 hr.

Flight experiments are in progress to assess the temperatures at which aphids cease to fly; an aphid fastened by gum on to a pin is allowed to fly in an air stream whose temperature is lowered gradually. The rate of cooling strongly affects the temperature at which flight ceases.

Aphids are weak fliers, therefore the direction of the wind determines their direction of travel. This is reflected in the intensity of infestation on the windward edges of fields.

Insect Rearing and Methods of Testing Insecticides. By the Staff of the Department of Insectides and Fungicides (communicated by K. A. LORD). (*Rothamsted Experimental Station, Harpenden, Herts*)

Satisfactory control of insect pests by chemicals is only possible if suitably toxic chemicals are available and can be administered economically in sufficient quantity without causing harm to man, crops or farm animals.

In practice insects may acquire a toxic dose of insecticide as a vapour, by ingestion of contaminated food or by contact with the substance, when it is absorbed through the cuticle.

A variety of laboratory techniques for the bioassay of insecticides is demonstrated. The equipment makes it possible to administer controlled doses of insecticides by various routes and under different conditions.

Potter tower. A device for applying a uniform film of insecticide over a 9 cm. Petri dish, mainly used for the comparison of the toxicity of contact insecticides either as direct sprays or residual films.

Dipping apparatus. Equipment devised to test changes in toxicity of large crystals broken up by spray nozzles. It can be used in other work on the action of contact insecticides.

Topical application. Measured drops of insecticide are placed on the insect cuticle and the toxicity of insecticides by direct application may be measured. This method has been used to investigate the synergistic activity of piperonyl butoxide and sulphoxide on the pyrethrins. This apparatus may also be used for the subcuticular injection of poisons.

Stomach poison tests. Equipment used for assessing the toxicity of substances ingested by insects, avoiding as far as possible contact action.

An adequate supply of healthy test insects is essential to bioassay and the rearing of some of the insects frequently used in the department is illustrated. These include:

Diataraxia oleracea (tomato moth); *Gryllus domesticus* (cricket); *Blattella germanica* (German cockroach); *Musca domestica* (house flies); *Blatta orientalis* (oriental cockroach); *Periplaneta americana* (American cockroach); *Acyrthosiphon pisum* (pea aphid); *Megoura viciae* (vetch aphid); *Dysdercus fasciatus* (cotton stainer); *Pieris brassicae* (cabbage whites); *Phaedon cochleariae* (mustard beetle); *Drosophila melanogaster* (fruit fly); *Ephestia kühniella* (grain moth); *Tenebrio molitor* (mealworm); *Oryzaephilus surinamensis* (saw-toothed grain beetle); *Tribolium castaneum* (red rust flour beetle); *Calandra oryzae* (rice weevil); *Achroia grisella* (wax moth); *Trogoderma granarium* (Khapra beetle); *Oryzaephilus mercator* (merchant grain beetle).

Insect pests may be controlled by destruction of the eggs by the application of chemicals. Frequently the embryos appear to develop normally but fail to hatch.

Studies on the toxicity of insecticides to eggs are illustrated in relation to the development of esterases.

A Model to Demonstrate Separation in Double Helices. By C. O. Person (introduced by H. S. A. Sherratt). (*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts*)

The model consists of three parallel rods, each of which is free to rotate. A double helix, formed of cotton tape wound about the centre rod with free ends attached one to each of the side rods, is unwound and two single helices are simultaneously formed when the three rods are rotated.

Deoxyribonucleic acid (DNA) is usually considered to have a molecular structure consisting of two complementary, helical polynucleotide chains related to a common axis (Watson & Crick, 1953). A difficulty encountered in the interpretation of DNA replication, supposed to occur in living cells, is in accounting for the separation of the two chains so that *each* chain may then act as a template for the formation of a new double helix. A process of rotation similar to that achieved by the model, if proposed for the reproduction of the DNA molecule, would require that the helix be free to rotate, and that complementary chains form on to, and impose, a helical structure on the separating DNA chains. This mechanism would be simpler than others that have been advanced previously (Delbrück, 1954; Gamow, 1955).

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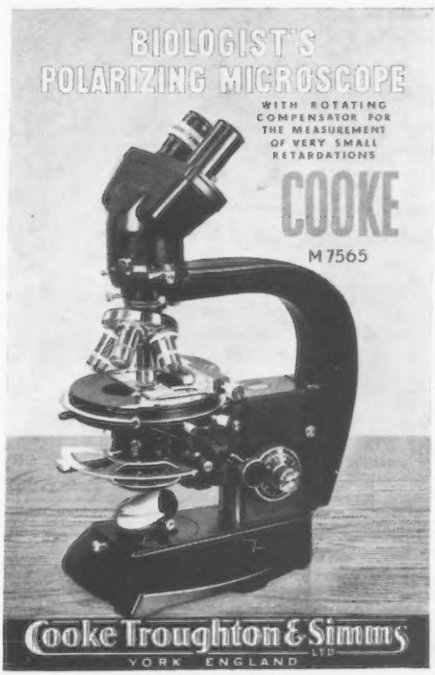

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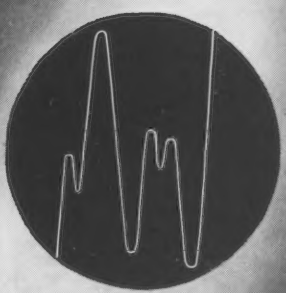
spectro  vision

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Azulene	42/- G
Batyl alcohol (from hydrogenated selachyl alcohol)	9/- G
Behenyl alcohol	7/- D
1, 12-Benzperylene (10 mgm amps)	25/- A
Benzyl acetone	21/- H
Benzyl ethyl ketone	65/- H
Benzyl mercaptan	30/- H
2, 5-Bis-ethyleneimino hydroquinone	11/- G
o-Bromo anisole	39/- H
m-Bromobenzotrifluoride (bp. 150–154° C)	28/- D
Bufotenin bioxalate	258/- G
n-Butyl-aniline	38/- K
p-tert-Butyl toluene	97/- K
Caesium (metal) (5 gram ampoules)	45/- G
o-Carboxyphenyl phosphate	49/- G
L-Carnosine (100 mgm ampoules)	57/- d
Cephaeline ($C_{28}H_{38}N_2O_4$)	19/- G
Chloro-difluoroacetic acid ($CClF_2.COOH$)	7/- D
2-Chloro-4-nitrobenzoic acid	10/- D
4-Chloro-*m*-phenylenediamine sulphate	21/- H
2-Chloro-*p*-phenylenediamine sulphate	19/- H
Chlorophyll a, chromatographically pure (10 mgm amps)	110/- A
Chlorophyll b, chromatographically pure (10 mgm amps)	110/- A
Choline pantothenate (25% D-pantothenic acid activity)	7/- D
Coronene	36/- d
cyclo-Octane (octamethylene) (bp. 148° C)	42/- D
cyclo-Octene (bp. 142° C)	52/- D
Cytidine-2′, 3′-cyclic phosphate, barium	107/- d
Deoxyadenosine	170/- G
Deoxycytidine hydrochloride	198/- G
Deoxyguanosine	200/- G
2, 7-Diaminofluorene	58/- G
2, 3-Diamino-naphthalene	92/- H
Diamyl phthalate	16/- K
Dibromo-difluoroethane ($CH_2Br.CBrF_2$)	6/- D
2, 4-Dibromophenol	92/- H
2, 6-Dibromophenol	92/- D
α, β-Dibromo-phenylethane	95/- H
2, 6-Dibromopyridine	49/- D
2, 5-Dibromothiophene	56/- H
Di-*tert*-butylfluorobenzene	22/- D
Dibutyl glycol phthalate	34/- K
Dibutyl sebacate	42/- K
α, α-Dichlorodiphenylmethane	45/- D
Dichloro-*mono*fluoracetic acid ($(CCl_2F.COOH$)	7/- D
Dichloro-fluoroacetic anhydride ($(CCl_2F.CO)_2O$)	15/- D
Dichloro-tetrafluoroacetone ($CClF_2.CO.CClF_2$)	9/- D
1, 4-Di-cyclohexylbenzene	58/- H
N, N′-Dicyclohexylcarbodi-imide	98/- H
2, 6-Diethyl aniline	23/- H
4, 4′-Difluorodiphenyl	18/- D
2, 3-Dihydropyran	43/- H
2, 3-Dihydroxyquinoxaline	46/- D
p-Di-iodobenzene	38/- D
2, 3-Dimercapto-propanol	38/- D
2, 4-Dimethyl-acetophenone (4-aceto-*m*-xylene)	25/- H
3-Dimethylamino-benzoic acid	79/- H
2, 3-Dimethyl-butylamine	7/- D
2, 5-Dimethyl-*cyclo*-hexanol	12/- H
3, 4-Dimethyl-*cyclo*-hexanol	18/- H
3, 5-Dimethyl-*cyclo*-hexanol	17/- H
Dimethyl glycol phthalate	26/- K
2, 5-Dimethyl-hexanediol	22/- H
2-Dimethyl-4-oxymethyl-1, 3-dioxolan	28/- K
2, 6-Dimethyl piperidine	35/- H
2, 6-Dimethyl-quinoline (mp. 57° C)	132/- H
1, 2, 3, 4-*peri*-Dinaphthylene-fluorene	45/- D
2, 5-Dinitrofluorene	15/- G
Dinonyl adipate	32/- K
Dinonyl sebacate	39/- K
Dioctyl adipate	37/- K
Dioctyl sebacate	41/- K
N, N-Disalicylidene ethylene diamine	93/- K
Ditetrazolium iodide	45/- G
2, 2′-Dithio dibenzoic acid ($C_{14}H_{10}O_4S_2$)	19/- H
Dithiodiglycol	21/- H
Dithiodipropanol	23/- H
N, N′-Di-*p*-tolyl carbodiimide	25/- D
Dodecyl aldehyde	75/- H
Dodecyl-*p*-hydroxy-benzoate	15/- H
n-Dodecyl mercaptan	96/- K
Dodecyl methacrylate	72/- K
Endomethylene tetrahydrophthalic anhydride	45/- K
Ergocornin, alkaloid	450/- G
Ergocryptin, alkaloid	460/- G
L-Ergothioneine hydrochloride	275/- G
Erucyl alcohol	7/- D
p-Ethoxy-acetophenone	65/- K
Ethyl acetylamino-malonate	43/- H
Ethylene-imine (10 gram ampoules)	32/- D
p-Ethyl phenol	35/- H
Ethyl sodium oxalacetate	24/- K
p-Fluorobenzene sulphonic acid	42/- D
p-Fluorobenzene sulphonyl chloride	56/- D
m-Fluorobenzotrifluoride (bp. 98-99° C)	28/- D
p-Fluorobenzotrifluoride (bp. 101-103° C)	26/- D
1-Fluoro-2-chlorobenzene	90/- H
1-Fluoro-3-chlorobenzene	80/- H
1-Fluoronaphthalene (bp. 212-214° C)	60/- D
2-Fluoronaphthalene	9/- G
p-Fluoro-nitrobenzene	12/- D
Galactosamine hydrochloride (100 mgm amps)	65/- d
Glycyl-DL-serine	68/- G
Gramine (β-dimethylaminomethyl indole)	11/- D
Hexadecanolid	115/- D
DL-Histidine dihydrochloride	35/- D
p-Hydroxy-azobenzene-*p′*-sulphonic acid	68/- D
β-Hydroxy ethyl hydrazine	53/- H
N-(2-Hydroxyethyl)-phenylacetamide	86/- K
5-Hydroxy-indolylacetic acid (10 mgm amps)	25/- A
1, 12-Hydroxy stearyl alcohol (95%)	19/- H
Indanol-4 (4-hydroxyhydrindene)	20/- H
Indanol-5 (5-hydroxyhydrindene)	21/- H
Indoxyl butyrate	35/- G
p-Iodo aniline	20/- D
m-Iodobenzotrifluoride (bp. 177° C)	31/- D
7-Iodo-8-hydroxyquinoline-5-sulphonic acid	31/- H
p-Iodo-phenol	72/- D
o-Iodo toluene	27/- H
p-Iodo toluene	38/- H

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